15:00:19 OCA PAD AMENDMENT - PROJECT HEADER INFORMATION 10/27/94

Active

Project #: E-19-X29 Cost share #: Rev #: 2
Center # : 10/24-6-R7829-0A0 Center shr #: OCA file #:
Work type : RES
Contract#: AGMT DTD 930622 Mod #: LTR DTD 7/29/94 Document : SUBCONT
Prime #: 1 P60 HL48482-01 Contract entity: GTRC

Subprojects ? : N CFDA: N/A
Main project #: PE #: N/A

Project unit: CHEM ENGR Unit code: 02.010.114
Project director(s):
WICK T M CHEM ENGR (404)894-8795

Sponsor/division names: EMORY UNIVERSITY / ATLANTA, GA
Sponsor/division codes: 400 / 012

Award period: 930415 to 950331 (performance) 950331 (reports)

Sponsor amount	New this change	Total to date
Contract value	103,592.00	201,852.00
Funded	103,592.00	201,852.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: THE MECHANISM OF SICKLE ERYTHROCYTE/ENDOTHELIAL ADHESION

PROJECT ADMINISTRATION DATA

OCA contact: Ina R. Lashley 894-4820

Sponsor technical contact

JAMES R. ECKMAN, M.D.
(404)589-3572

DEPARTMENT OF MEDICINE
EMORY UNIVERSITY
69 BUTLER ST.
ATLANTA, GA 30303

Sponsor issuing office

JANE O'CONNER
(404)727-2503

OFFICE OF SPONSORED PROGRAMS
EMORY UNIVERSITY
1462 CLIFTON RD., NE
ATLANTA, GA 30322

Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : N/A N/A supplemental sheet
Equipment title vests with: Sponsor X GIT
NONE PROPOSED. (SEE SUBGRANT ARTICLE 12 FOR REFERENCE.)
Administrative comments -
EMORY LTR DTD 7/29/94 AUTHORIZES YEAR 02 FUNDS OF $103,592 FOR THE PERIOD
4/1/94 - 3/31/95. *NOTE: THIS PROJECT IS "REOPENED"*

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 11/16/95
Original Closeout Started 05/24/94

Project No. E-19-X29_____ Center No. 10/24-6-R7829-0A0_

Project Director WICK T M_____ School/Lab CHEM ENGR_____

Sponsor EMORY UNIVERSITY/ATLANTA, GA_____

Contract/Grant No. AGMT DTD 930622_____ Contract Entity GTRC

Prime Contract No. 5 P60 HL48482-03_____

Title THE MECHANISM OF SICKLE ERYTHROCYTE/ENDOTHELIAL ADHESION_____

Effective Completion Date 950331 (Performance) 950331 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	950616
Final Report of Inventions and/or Subcontracts	Y	_____
Government Property Inventory & Related Certificate	N	_____
Classified Material Certificate	N	_____
Release and Assignment	N	_____
Other _____	N	_____

Comments_____

Subproject Under Main Project No. _____

Continues Project No. _____

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other _____	N
_____	N

E-19-X-29

GEORGIA TECH RESEARCH CORPORATION

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
PROGRAM INITIATION DIVISION
ATLANTA, GEORGIA 30332-0420
USA

Telex: 542507 GTRC OCA ATL
Fax: (404) 894-6956

Phone: (404) 894-4817

Refer to: DB/02.400.012.94.003

5 January 1994

Emory University
80 Butler Street
15 A Sickle Cell
Atlanta, Ga 30335

Attention: Dr. James Eckman

Subject: Research Proposal Entitled, "Georgia Comprehensive Sickle Cell Center - Project 1"

Ladies and Gentlemen:

The GEORGIA TECH RESEARCH CORPORATION desires to submit for your consideration the subject proposal prepared by Dr. Timothy M. Wick, School of Chemical Engineering, Georgia Institute of Technology.

A description of the research program, the time required and program cost are included in the proposal. Should additional information be desired, please do not hesitate to contact Dr. Wick at 404/894-8795 regarding technical matters or the undersigned at 404/894-4817 for administrative concerns.

In the event of an award, we propose that the work be authorized by either a grant or a cost-reimbursable (no-fee) type contract drawn in the name of the GEORGIA TECH RESEARCH CORPORATION.

We appreciate the opportunity of submitting this proposal and look forward to working with you on this project.

Sincerely,

David B. Bridges
Contracting Officer

DBB/kgw

Addressee: Three copies
Enclosure: Proposal - Three copies

Form approved through 6/30/94
OMB No. 0925-0001

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

APPLICATION FOR CONTINUATION GRANT

REVIEW GROUP	TYPE	ACTIVITY	GRANT NUMBER
			1P60 HL48482-02

TOTAL PROJECT PERIOD
From: 1 April 1993 Through: 31 March 1998

REQUESTED BUDGET PERIOD
From: 1 April 1993 Through: 31 March 1994

1. TITLE OF PROJECT
Georgia Comprehensive Sickle Cell Center - Project #1

2a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR *(Name and address, street, city, state, zip code)*

Timothy M. Wick, Ph.D.
School of Chemical Engineering
Georgia Institute of Technology
778 Atlantic Drive
Atlanta, GA 30332-0100

4. APPLICANT ORGANIZATION *(Name and address, street, city, state, zip code)*

Georgia Tech Research Corp.
OCA/PID Rm. 246 CRB
Georgia Institute of Technology
Atlanta, GA 30332-0420

BITNET/INTERNET ADDRESS
timothy.wick@che.gatech.edu

5. ENTITY IDENTIFICATION NUMBER
1580603146A1

2b. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT
School of Chemical Engineering

2c. MAJOR SUBDIVISION
College of Engineering

6. TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL
Office of Contract Administration
Georgia Institute of Technology
Atlanta, GA 30332-0420

3. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR BIOMEDICAL RESEARCH SUPPORT GRANT *(See instructions)*

20 other

BITNET/INTERNET ADDRESS

7. HUMAN SUBJECTS — If "YES" exemption no. or IRB approval date — 4b. Assurance of compliance no.

[ ] 7a. NO [X] YES — M1395

8. VERTEBRATE ANIMALS — If "YES," IACUC approval date — 8b. Animal welfare assurance no.

[X] 8a. NO [ ] YES

10. COSTS REQUESTED FOR NEXT BUDGET PERIOD
10a. DIRECT $80,144 10b. TOTAL $ 109,797

11. INVENTIONS AND PATENTS *(See instructions)*
[ ] NO [ ] YES If "YES," [ ] Previously reported [ ] Not previously reported

9. PERFORMANCE SITE(S) *(Organizations and addresses)*

Georgia Institute of Technology
Cellular Adhesion Laboratory
Space Science and Technology Building A
Room 217
Atlanta, GA 30332-0405

TELEPHONE AND FAX INFORMATION

	AREA CODE	TELEPHONE NO. AND FAX NO.
12a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR *(Item 2a)*	404 404	894-2795 894-2866
12b. NAME OF ADMINISTRATIVE OFFICIAL *(Item 6)* David B. Bridges	404 404	894-6956 (fax) 894-4817
12c. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION *(Item 15)* David B. Bridges Contracting Officer		

BITNET/INTERNET ADDRESS david.bridges@oca.gatech.edu

13. DO NOT USE THIS SPACE

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application. Willful provision of false information is a criminal offense (U.S. Code, Title 18, Section 1001). I am aware that any false, fictitious, or fraudulent statement may, in addition to other remedies available to the Government, subject me to civil penalties under the Program Fraud Civil Remedies Act of 1986 (45 CFR 79).	SIGNATURE OF PERSON NAMED IN 2a *(In ink. "Per" signature not acceptable.)*	DATE 1/4/94
15. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with the Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense (U.S. Code, Title 18, Section 1001). I am aware that any false, fictitious, or fraudulent statement may, in addition to other remedies available to the Government, subject me to civil penalties under the Program Fraud Civil Remedies Act of 1986 (45 CFR 79).	SIGNATURE OF PERSON NAMED IN 12c *(In ink. "Per" signature not acceptable.)*	DATE 1/5/94

DETAILED BUDGET FOR NEXT BUDGET PERIOD DIRECT COSTS ONLY	FROM 1 April 1994	THROUGH 31 March 1995	GRANT NUMBER 1P60 HL48482-02

PERSONNEL (Applicant organization only) NAME	ROLE ON PROJECT	TYPE APPT. (months)	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED (Omit cents) SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Timothy M. Wick, Ph.D.	PI	12	20	70,140	14,028	3,521	17,549
James R. Eckman, M.D.	Co-PI	12	5				
Paula Smolinski	Graduate Student	12	100	22,500	22,500	----	22,500
R. Montez	Graduate Student	12	100	22,500	22,500	----	22,500
SUBTOTALS →							62,549

CONSULTANT COSTS

EQUIPMENT *(Itemize)*

None.

SUPPLIES *(Itemize by category)*

Tissue culture media, fetal bovine serum, EC growth factors adhesive proteins, buffers, antibiotics	$ 6,600
Disposable plasticware (LabTek chambers, pipets, flasks, filters, gloves)	$14,000
Monoclonal antibodies, synthetic peptides	$ 2,000
Electrophoresis supplies, ELISA Reagents	$ 1,363
	13,963

TRAVEL

Travel to two scientific meetings for investigators — 1,560

PATIENT CARE COSTS — INPATIENT

OUTPATIENT

ALTERATIONS AND RENOVATIONS *(Itemize by category)*

None.

OTHER EXPENSES *(Itemize by category)*

Publication fees, artwork, photography	($1,200)
Machine Shop for manufacture of flow loops	($ 872)
	2,072

SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD

DIRECT COSTS $ 80,144

INDIRECT COSTS $ 29,654 (37%) TOTAL → 29,653

TOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD *(Enter on Page 1, Item 10a)* → $ Direct $80,144 Total $109,797

BUDGET JUSTIFICATION	GRANT NUMBER 1P60 HL48482-02

SUPPLEMENTAL INFORMATION REGARDING *ITEMS* IN THE PROPOSED BUDGET FOR THE NEXT PERIOD WHICH REQUIRE EXPLANATION OR JUSTIFICATION. *(See instructions)*

Personnel: Fringe benefits are 25.1% of salary.
Principal Investigator - Dr. Timothy M. Wick, Ph.D.: Funding is requested for the Principal Investigator to provide time to organize the study, coordinate *in vitro* investigations with clinical studies, perform experiments, analyze data, prepare manuscripts, hold regular laboratory meetings of the investigators, prepare manuscripts, and develop progress reports. It is estimated that 20% of Dr Wick's time will be devoted to these tasks related to the Project #1 of the Georgia Comprehensive Sickle Cell Center.
Graduate Student - Paula Smolinski: Ms. Smolinski has been working in the laboratory since September 1992. She has recently begun doing sickle cell adherence studies and is responsible for much of the new data presented in the Results section. Ms. Smolinski will devote 100% of her effort to this project. Ms. Kumar is (and will continue to be) responsible for the adhesion assays related to $\alpha 4\beta 1$/VCAM-1 mediated adherence, endothelial cell activation with viruses, and the potential relationship(s) between infection, sickle cell adherence, and vaso-occlusive pain events.
Graduate Student - Richard Montez: Mr. Montez is a new graduate student who will replace Mr. Henri Britain who recently earned his Ph.Dßœ. Mr. Montez is currently designing novel flow chambers to more accurately determine the role between fluid mechanical forces (shear stress), endothelial cell function, and adherence. These studies will be invaluable to develop new studies which more closely account for the complex fluid dynamics *in vivo*.

Supplies: Tissue culture costs are based upon current performance of 2 flow experiments per week as well as current usage and costs. Media, serum, growth factors, buffers, and other chemicals as well as plasticware, glassware, and gloves are required for cell cultures and adhesion assays. Monoclonal antibodies to adhesion receptors will be used to identify receptors that are necessary for sickle erythrocyte adhesion to endothelium.
Travel: Funds are requested for Dr. Wick or an associate to attend ASH and the annual Meeting of the Sickle Cell Disease Program to present research and interact with colleagues interested in similar and related areas of hematology and sickle cell anemia.
Other Expenses: Funds are requested to cover the cost photocopying and postage related to the transfer of data and data forms between Emory, Grady and Georgia Tech, medical illustrations and page costs. Machine shop charges are required to construct new adhesion systems.

CURRENT BUDGET PERIOD	FROM	THROUGH
	1 April 1994	31 April 1995

The following pertains to your CURRENT PHS budget. This information may be used in determining the amount of support for the NEXT budget period.

A. CURRENT BUDGET	TOTAL ESTIMATED EXPENDITURES AND OBLIGATIONS (1)	ESTIMATED UNOBLIGATED BALANCE (2)	EXPLAIN ANY SIGNIFICANT ESTIMATED UNOBLIGATED BALANCE IN COLUMN 2 (3)
TOTAL DIRECT COSTS	67,812	0	
INDIRECT COSTS *(As provided)*	30,448	0	
TOTALS →	**98,260**	0	None

GRANT NUMBER: _____

BIOGRAPHICAL SKETCH

Give the following information for all new key personnel, consultants, and collaborators.
Copy this page for each person.

NAME	POSITION TITLE
Timothy M. Wick	Assistant Professor

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Colorado, Boulder, CO	B.S.	1983	Chemical Engineering
Rice University, Houston, TX	Ph.D.	1988	Chemical Engineering
Rice University, Houston, TX	Post-Doc	1988	Biochemistry and Chemical Engineering

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individual who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience

9/88-present Assistant Professor, School of Chemical Engineering, Georgia Institute of Technology, Atlanta, GA
4/93-present Adjunct Assistant Professor, School of Mechanical Engineering, Georgia Tech, Atlanta, GA
2/88-9/88 Post-doctoral Research Associate, Department of Chemical Engineering, Rice University, Houston, TX
2/88-9/88 Post-doctoral Research Associate, Department of Chemical Engineering, Rice University, Houston, TX

Honors and Awards

1993 Ad-Hoc Reviewer. Academic Research Initiation Grants Program, North Carolina Biotechnology Center.
1993 Ad-Hoc Reviewer. Biomaterials Panel, Biomedical Engineering and Research to Aid Persons with Disabilities Program, National Science Foundation .
1993 Member. Special Emphasis Panel, NIH Collaborative Projects (RO1s) on Minority Health.
1993 Reviewer. Biomedical Engineering Society 1993 Graduate Student Awards Committee.
1993 Outstanding Chemical Engineering Professor. Awarded by the Student Chapter of Omega Chi Epsilon.
1992 Lilly Foundation Teaching Fellowship.
1991 Young Investigator Award Finalist. The 1991 World Congress on Medical Physics and Biomedical Engineering.
1991 American Heart Association-Georgia Affiliate, Grant-In-Aid
1991 The Whitaker Foundation, Biomedical Engineering Research Grant
1991 NIH-First Independent Research and Transition (FIRST) Award
1990, 91 Du Pont Young Faculty Award
1989 American Heart Association-Georgia Affiliate, Grant-In-Aid
1987 Beecham Award for outstanding original research presented at annual meeting of the Southern Society for Clinical Investigation, the Southern Section of the AFCR and the Southern Society for Pediatric Research
1986 Omega Chi Epsilon (National Chemical Engineering Honor Society)

Original Articles

1. Wick, T.M., J.L. Moake, M.M. Udden, S.G. Eskin, D.A. Sears and L.V. McIntire. "Unusually Large von Willebrand Factor Multimers Increase Adhesion of Sickle Erythrocytes to Endothelial Cells Under Controlled Flow." Journal of Clinical Investigation, 80:905-910 (1987).
2. Wick, T.M., S.D. Doty, and R.M. Nerem. "Influence of Fluid Mechanical Stresses on Vascular Cell Adhesion." In: Biomechanical Transport Processes, F. Mosora, C. Caro, E. Krause, H. Schmid-Schönbein, C. Baquey, and R. Pelissier, eds, Plenum, New York, pp. 283-292, 1990.
3. Wick, T.M. and V. Louis. "Cytoadherence of *Plasmodium falciparum*-Infected Erythrocytes to Human Umbilical Vein and Human Dermal Microvascular Endothelial Cells under Shear Conditions." Am J Tropical Med Hygiene 45: 578-586 (1991).
4. Swerlick, R.A., K. Lee, T.M. Wick, and T.J. Lawley. "Human Dermal Microvascular Endothelial but not Human Umbilical Vein Endothelial Cells Express CD36 *In Vivo* and *In Vitro*." Journal of Immunology, 148:78-83 (1992).
5. Yoganathan A.P., T.M. Wick, & H. Reul. "The Influence of Flow Characteristics of Prosthetic Valves on Thrombus Formation." In: Thrombosis, Embolism, and Bleeding. E.G. Butchart and E. Bodnar, eds, ICR Publishers, London, pp. 123-148, 1992.

6. Wick, T.M. and V. Louis. "*Plasmodium fragile*: Cytoadherence of Parasitized Rhesus Monkey Erythrocytes to Human Endothelial Cells under Shear Flow Conditions." Experimental Parasitology, **74**:228-231 (1992).
7. Brittain, H.A., J.R. Eckman, and T.M. Wick. "Sickle Erythrocyte Adherence to Large Vessel and Microvascular Endothelium under Physiologic Flow is Qualitatively Different." J Lab Clin Med, **19**:538-545 (1992).
8. Wick, T.M., J.L. Moake, M.M. Udden, and L.V. McIntire. "Unusually Large von Willebrand Factor Multimers Preferentially Promote Young Sickle and Non-sickle Erythrocyte Adhesion to Endothelial Cells," Am J Hematology, **42**:284-292 (1993).
9. Johnson, J.K., R.A. Swerlick, P. Millet, K. Grady, T.M. Wick. "Cytoadherence of *Plasmodium falciparum*-Infected Erythrocytes to Microvascular Endothelium is Regulatable by Cytokines and Phorbol Ester," J Infectious Diseases, **167**:698-703 (1993).
10. Brittain, H.A., J.R. Eckman, R.J. Howard, and T.M. Wick. "Thrombospondin from Activated Platelets Promotes Sickle Erythrocyte Adherence to Human Microvascular Endothelium under Physiologic Flow: A Potential Role for Platelet Activation in Sickle Cell Vaso-occlusion," Blood, **81**:2137-2143 (1993).
11. Flaherty, A.L. and T.M. Wick. "Prolonged Contact with Blood Alters Surgical Gown Permeability," The American Journal of Infection Control, **21**:249-256 1993.
12. Swerlick, R.A., J.R. Eckman, A. Kumar, M. Jeitler, and T.M. Wick. "α_4/β_1-Integrin Expression on Sickle Reticulocytes: Vascular Cell Adhesion Molecule-1-Dependent Binding to Endothelium," Blood, **82**:1891-99 (1993).
13. Wick, T.M., H.A. Brittain, R. Howard, and J.R. Eckman. "Thrombospondin from Activated Platelets Promotes Sickle Erythrocyte Adherence to Human Microvascular Endothelial Cells via CD36 and integrin receptors," In: Vascular Endothelium: Physiological Basis of Clinical Problems II, J. Catravas, A. Callow, N. Gillis, U. Ryan, A. Mantovani, and M. Yacoub, eds, Plenum, New York, (**In press**) 1994.

Published Abstracts (selected from greater than 50)

1. Wick, T. M., L. V. McIntire, M. M. Udden, D. A. Sears, S. G. Eskin and J. L. Moake. "Unusually Large von Willebrand Factor Multimers Increase Adhesion of Sickle Erythrocytes to Endothelial Cells Under Controlled Flow," Clinical Research, 35:603a,;1987.
2. Wick, T. M., L. V. McIntire, M. M. Udden, D. A. Sears, S. G. Eskin and J. L. Moake. "Unusually Large von Willebrand Factor Multimers Increase Adhesion of Sickle Erythrocytes to Endothelial Cells Under Controlled Flow," Clinical Research, 35:16a:1987.
3. Wick, T. M., J. L. Moake, M. M. Udden and L. V. McIntire. "Similarities Between Young Non-Sickle Erythrocyte and Sickle Erythrocyte Adhesion to Human Endothelial Cells: Evidence that Young Red Cells Contain Receptors for von Willebrand Factor Multimers," Clinical Research, **36**:370a;1988.
4. Wick, T. M., J. L. Moake, M. M. Udden, and L. V. McIntire. "Unusually Large von Willebrand Factor Multimers Bind to Glycoprotein Ib-like and Integrin Receptors on Sickle and Young Non-Sickle Erythrocytes and Endothelial Cells: A Mechanism for Sickle and Other Young Erythrocyte Adhesion to Endothelial Cells," Blood, **72**:76a;1988.
5. Brittain, H. A., T. M. Wick, and J. R. Eckman. "Abnormal Adhesion of Sickle Red Blood Cells to Human Microvascular Endothelial Cells: A Potential Role for the Plasma Milieu in the Initiation of Vaso-occlusion," Annals of Biomedical Engineering. **19**:580:1991.
6. Wick, T. M., H. A. Brittain, and J. R. Eckman. "Adherence of Sickle Erythrocytes to Cultured Endothelial Cells Under Shear Flow: Influence of Endothelial Origin on the Mechanism of Adherence," 1991 Advances in Bioengineering, Winter Annual Meeting of the ASME, BED Vol. **20**:81-84;1991.
7. Wick, T.M., H. A. Brittain, R. A. Swerlick, J. R. Eckman. "Thrombospondin from Activated Platelets Promotes Sickle Erythrocyte Adherence to Endothelium: A Potential Role for Platelet Activation in Sickle Cell Disease," Blood, **80**:76a;1992.
8. Wick, T. M., J. R. Eckman, A. Kumar, M. Jeitler, R. A. Swerlick. "Reticulocytes from Patients with Sickle Cell Anemia Express the $\alpha_4\beta_1$ Integrin Complex and Bind to TNF-α Activated Endothelial Cells via a VCAM-1/$\alpha_4\beta_1$ Dependent Mechanism," Blood, **80**:11a;1992.
9. Wick, T. M., P. A. Smolinski, M. K. Offermann, and J. R. Eckman. "Synthetic Double-Stranded RNA Increases Adherence of Sickle Red Blood Cells to Human Umbilical Vein Endothelial Cells via $\alpha_4\beta_1$ - Vascular Cell Adhesion Molecule-1 Pathway," Blood, **82**:352a (1993).
10. Kumar, A., J. R. Eckman, R. A. Swerlick, and T. M. Wick. "Stimulation of Sickle Erythrocytes with Phorbol Ester Promotes Adherence to Endothelium: A Potential Role for Activated VLA-4 on Sickle Reticulocytes," Blood, **82**:352a (1993).
11. Kumar, A., J. R. Eckman, and T. M. Wick. "Plasma Enhancement of Sickle Red Blood Cell Adherence to Microvascular Endothelial Cells Mediated by Integrin Receptors can be Inhibited by Conformationally Constrained RGD Peptides," Blood, **82**:353a (1993).
12. T.M. Wick, T. M,. M. D. Brown, and J. R. Eckman. "Sickle Red Blood Cells Induce Expression of Cell Adhesion Molecules on Human Umbilical Vein Endothelial Cells," Blood, **82**:352a (1993).

OTHER SUPPORT

(Use continuation pages if necessary)

GRANT NUMBER

1P60 HL48482-02

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to significant delays in the review and/or funding of the application.

Other support is defined as all funds or resources, whether Federal, non-Federal, or institutional, available to the principal investigator/program director (and other key personnel named in the application) in direct support of their research endeavors through research or training grants, cooperative agreements, contracts, fellowships, gifts, prizes, and other means.

Reporting requirements are: For each of the key personnel, describe (1) all currently ***active*** support and (2) all applications and proposals ***pending*** review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check "None." Use continuation pages as needed to provide the required information in the ***format*** as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

Name Timothy M. Wick, Ph.D. Active X Pending _____ None _____

a. Source and identifying no. The Whitaker Foundation P.I. Timothy M. Wick

Title THe Role of Hemodynamics and Endothelial Cell Activation in Atherosclerosis

b. Your role on project Principal Investigator % Effort 15%

c. Dates and costs of entire project 1 July 1991 - 30 June 1994 ($179,999)

d. Dates and costs of current year 1 July 1993 - 30 June 1994 ($49,937 direct)

e. Specific aims of project To identify receptor-mediated pathways utilized by monocytes to adhe:

to vascular endothelium.

f. Describe scientific and budgetary overlap None.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

None.

OTHER SUPPORT.

Dr. Wick As PI:

1a. Source and Identifying Number: National Institutes of Health. 1R29 HL44960.
Title: The Mechanism of Sickle Erythrocyte/Endothelial Adhesion
b. Your role on project: Principal Investigator
% Effort: 50%
c. Dates and costs of entire project: 25 July 1991-30 June 1996 $349,992 (direct)
d. Dates and costs current year: 1 July 1993 - 30 June 1994 $67,005 (direct)
e. Specific aims of project: The aims of this project are to (1) characterize the difference in adhesion of sickle erythrocytes to endothelial cells from microvascular and umbilical vein endothelial cells by determining the effects of agonists and antagonists on adherence, (2) determine the variability in sickle red cell adhesion to endothelial cells from patient to patient and for individual patients during crisis and asymptomatic periods, (3) identify plasma factors responsible for adherence, (4) contrast the effects of agonists which presumably stimulate endothelial cells, such as thrombin, endotoxin, plasmin, histamine, fibrin(ogen), interleukin-1, and tumor necrosis factor on adherence. The R29 research involves different graduate research assistants, and although complementary, is separate from the SCORE research.
f. Describe budgetary and scientific overlap: None.
g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.): None.

Pending

1a. Source and Identifying Number: AHA - Georgia Affiliate
Title: The Mechanism of Shear-Induced Monocyte Adherence to Endothelium: Implications for Atherosclerosis
b. Your role on project: Principal Investigator **% Effort:** 5%
c. Dates and costs of entire project: 1 July 1994-30 June 1996 $65,992 (total)
d. Dates and costs current year: 1 July 1994 - 30 June 1995 $32,996 (total)
e. Specific aims of project: This research will elucidate the mechanisms of shear stress induced endothelial cell expression of VCAM-1 and its role in monocyte recruitment in atherogenic lesions.
f. Describe budgetary and scientific overlap: None.
g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.): None.

Dr. Wick as Co-investigator:

1a. Source and Identifying Number: NIH 1-PO1-HL48482-01 Project 2
P.I. Dr. James R. Eckman, M.D., Professor of Medicine in Hematology/Oncology, Emory University School of Medicine, Atlanta, Georgia is PI for the Comprehensive Sickle Cell Center, Dr. Robert Nerem, Ph.D., Professor, School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA is PI for Project #2.
Title: Influence of Sickle Erythrocytes on Endothelial Biology
b. Your role on project: Collaborating Investigator on Project #2 **% Effort:** 10%
c. Dates and costs of entire project: 1 April 1993-31 March 1998 $597,671 (total)
d. Dates and costs current year: 1 April 1993 - 31 March 1994 $106,862 (total)
e. Specific aims of project: This project elucidates the effects of sickle erythrocytes on endothelial cell structure and function. Specifically the influence of sickle cells on endothelial cell viability, morphology, growth factor secretion, and thrombogenicity is being investigated. These studies are aimed at determining the role of sickle erythrocyte membranes in vascular pathologies, particularly those associated with large vessel pathologies not likely associated with sickle cell adherence and microvascular occlusion. Dr. Wick , as a co-investigator draws 10% salary from this grant and contributes 10% effort.
f. Describe budgetary and scientific overlap: None.
g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.): None

2a. Source and Identifying Number: NIH-NRSA NIGMS GM08433
P.I. Robert M. Nerem, Ph.D. Professor, School of Mechanical Engineering, Georgia Tech
Title: Cellular Engineering Training Grant
b. Your role on project: Collaborating Faculty
% Effort: 0%
c. Dates and costs of entire project: $339,208 (26 September 1991 - 31 August 1995)
d. Dates and costs current year: 1 September 1993 - 31 August 1994
e. Specific aims of project: This project provides funds for 4 predoctoral students studying Cellular Engineering. Dr. Wick supervises one of these students working on sickle cell/endothelial cell interactions.
f. Describe budgetary and scientific overlap: None.
g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.): None.

3a.Source and Identifying Number: The Whitaker Foundation
P.I. Robert M. Nerem, Ph.D. Professor, School of Mechanical Engineering, Georgia Tech
Title: Biomedical Engineering Education: An Interdisciplinary Tissue Engineering Education and Research Program
b. Your role on project: Participating faculty
% Effort: 0%
c. Dates and costs of entire project: $3,000,000 (1 September 1993 - 31 August 1996)
d. Dates and costs current year: $1,000,000 (1 September 1993 - 31 August 1994)
e. Specific aims of project: This grant provides funds for laboratory space renovation, the hiring of six new faculty in Tissue Engineering, and a limited number of graduate student stipends.
f. Describe budgetary and scientific overlap: None.
g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.): None.

4a. Source and Identifying Number: NSF BCS-9111761
P.I. Robert M. Nerem, Ph.D. Professor, School of Mechanical Engineering, Georgia Tech
Title: Reconstitution of a Vascular Graft
b. Your role on project: Co-investigator
% Effort: 5%
c. Dates and costs of entire project: $535,732 (1 September 1991 - 28 February 1995)
d. Dates and costs current year: $150,000 (1 September 1993 - 31 August 1994)
e. Specific aims of project: Dr. Wick will evaluate the thrombogenicity of tissue engineered blood vessels developed in Dr. Nerem's lab.
f. Describe budgetary and scientific overlap: None. Dr. Wick will spend approximately $15,000 of this grant in the final year to investigate platelet adherence.
g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.): None.

Pending Applications

1a.Source and Identifying Number: NSF-Engineering Research Center
P.I. Robert M. Nerem, Ph.D. Professor, School of Mechanical Engineering, Georgia Tech
Title: A Research Center for the Engineering of Living Tissues
b. Your role on project: Participating faculty
% Effort: 5%
c. Dates and costs of entire project: $11,048,605 (1 September 1994 - 31 August 1999)
d. Dates and costs current year:
e. Specific aims of project: This grant application is to develop a center at Georgia Tech to develop research and new technology for the engineering of new cardiovascular devices.
f. Describe budgetary and scientific overlap: None.
g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.): None.

1. Specific Aims

The tendency for hemoglobin SS to polymerize at low oxygen tension is assumed to dominate sickle cell pathology. Since morphological sickling is delayed after hemoglobin deoxygenation, factors which delay erythrocyte microcirculatory transit are likely antecedents to microvascular occlusion, ischemic tissue damage, and pain episodes characteristic of sickle cell anemia. Our central hypothesis is that sickle erythrocyte adherence to microvascular endothelium delays erythrocyte microcirculatory transit permitting intracapillary erythrocyte sickling leading to complete occlusion (1). Adherence may be affected by secondary perturbations in hematological parameters such as infection which can activate the endothelium to favor adherence. Our data clearly indicate that plasma, red cell and endothelial cell factors, and hemodynamics all likely contribute to adherence and occlusion *in vivo* (2-7). Our **specific aims for this project** are to (i) demonstrate that infection induces endothelial cell alterations which increase sickle erythrocyte adherence, (ii) quantify the expression of endothelial cell ICAM-1, VCAM-1, and E-selectin after exposure to sickle erythrocytes and determine the mechanism of sickle red cell adherence to erythrocyte-activated endothelium, (iii) develop novel adhesion systems to study the interrelationship between adherence, endothelial cell function, and fluid mechanics under conditions which closely mimic those *in vivo*. It is becoming clear that red cells, white cells, endothelium and hemodynamics interact to alter cellular function. These alterations are exacerbated by the presence of sickle hemoglobin. The long-term goal of this research, in close collaboration with Projects 2,3, and 4 is to elucidate the role of red cells, endothelium, and hemodynamics as well as the extent to which the thrombotic and immune systems participate in sickle cell vascular complications.

2. Studies and Results

Elucidate the role of infection in sickle cell vascular complications

Anecdotal evidence suggests that infection may initiate or participate in vascular complications associated with sickle cell anemia (8-10) although the possible relationship between infection and vascular complications in sickle cell anemia has not yet been established. Since sickle reticulocytes adhere to cytokine activated (e.g. VCAM-1 expressing [14]) endothelium via a VCAM-1/$\alpha_4\beta_1$-dependent mechanism (11) and double-stranded RNA (a model for viral infection [12]) induces endothelial cell expression of VCAM-1 (13), we hypothesized that double-stranded RNA induction of endothelial VCAM-1 would increase sickle erythrocyte adherence via a similar mechanism. When endothelial cells were incubated with the synthetic double stranded RNA poly (I:C) (polyinosinic acid:polycytidilic acid) endothelial cell VCAM-1 expression increased (Fig 1) leading to a concomitant increase in sickle, but not normal erythrocytes (Fig 2) under continuous flow conditions at a shear stress of 1.0 dyne/cm^2. This increase in VCAM-1 expression and adherence was not observed when endothelial cells were incubated with single stranded RNA (Poly (I)) (Figure 2). Preincubation of endothelium with anti-VCAM-1 antibody or erythrocytes with anti-$\alpha_4\beta_1$ antibody inhibited Poly (I:C) induced sickle

erythrocyte adherence by 100% and 56%, respectively. Neither anti-glycophorin on the erythrocytes or anti-ICAM-1 on the endothelium inhibited the adherence induced by Poly (I:C) (not shown).

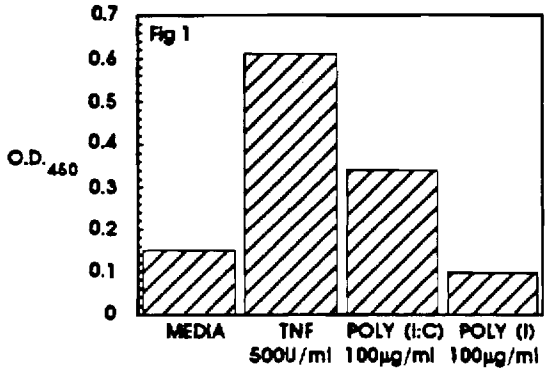

Figure 1. Poly (I:C) induces VCAM-1 expression. ELISA data (15) after indicated incubation for 22 hours. Data are representative experiment performed on four separate occasions.

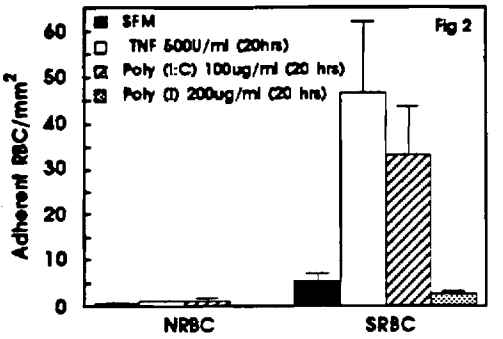

Figure 2. Sickle erythrocytes adhere to poly (I:C) stimulated endothelium. Data are mean±S.D. of experiments with blood from 11 homozygous, asymptomatic sickle patients and 5 normal donors.

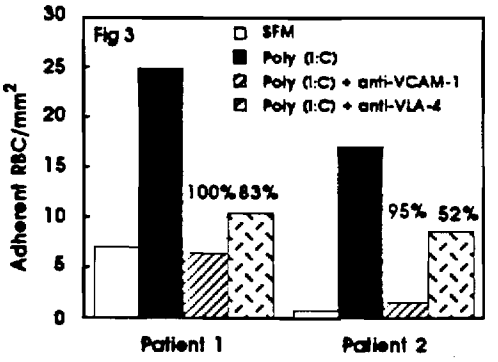

Figure 3. Anti-VCAM-1 or anti$_{\alpha}\beta_1$ antibody inhibits sickle erythrocyte adherence to Poly (I:C) activated endothelium. Data are two representative patients of five studied to dat

Quantification of expression of endothelial cell adhesion molecules induced by sickle erythrocytes

The above data suggest that a variety of factors can stimulate endothelial cell adhesivity and increased sickle erythrocyte adherence. Others have demonstrated that some vascular lesions in sickle cell anemia are localized to large cerebral arteries and lead to increased incidence of large vessel complications, including stroke and other neurological complications, in sickle patients (16-18). Clearly these lesions are not the result of microvascular occlusion. Since these clinical complications can apparently be eliminated with transfusion therapy, we hypothesize that sickle erythrocyte membrane abnormalities can directly activate endothelium, leading to increased red cell (and possibly white cell or platelet) adherence. As can be seen in Figures 4-6, incubation of cultured human umbilical vein endothelium with

sickle erythrocytes increases endothelial expression of VCAM-1, ICAM-1, and E-selectin.

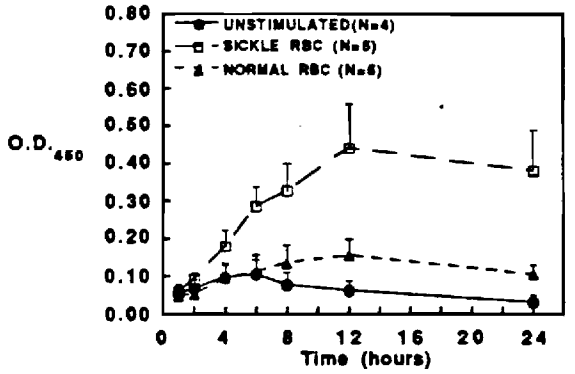

Figure 4. Time course of VCAM-1 upregulation. Data are mean±SEM of indicated number of experiments. Washed sickle or normal erythrocytes were incubated at 1% hematocrit with confluent monolayers of endothelium for the indicated times.

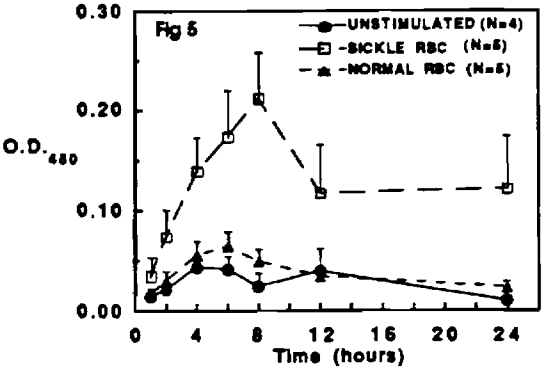

Figure 5. Time course of E-selectin upregulation.

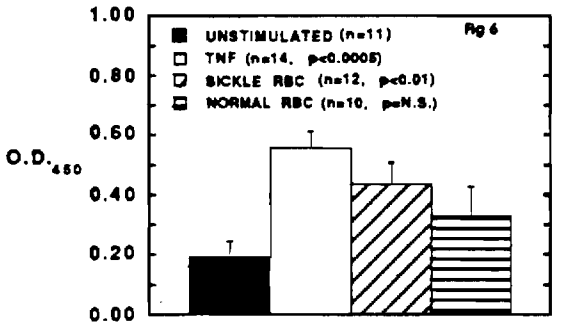

Figure 6. Time course of ICAM-1 upregulation.

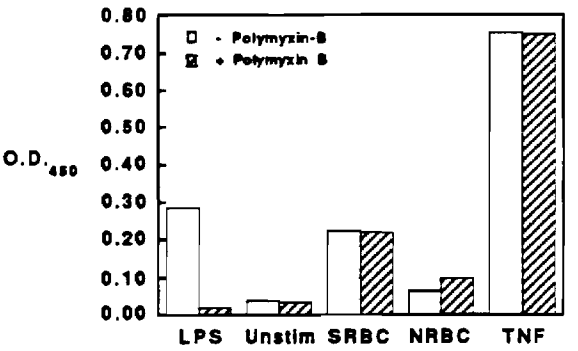

Figure 7. Polymyxin B does not inhibit sickle erythrocyte-induced endothelial cell adhesion molecule expression.

The sickle erythrocyte induction of endothelial cell adhesion molecule expression is not related to possible bacterial contamination since polymyxin B, a potent inhibitor of lipopolysaccharide induced VCAM-1 expression, had no effect on the observed phenomena (Figure 7).

Finally, since sickle erythrocytes can induce endothelial cell VCAM-1 (and other cell adhesion molecule expression), studies were undertaken determine whether endothelium exposed to sickle red cells could support higher adherence of sickle erythrocytes. For these studies, human umbilical vein endothelial cells were incubated with washed sickle erythrocytes at 1% hematocrit for 8 hours. Then, the sickle red cells were removed from the endothelial cells by washing for 20 minutes at 1 dyne/cm^2. Adherence assays were then performed with fresh washed erythrocytes from the same donor. As

can be seen in Figure 8, sickle red cell adherence was elevated on endothelial cell monolayers pre-exposed to sickle red cells.

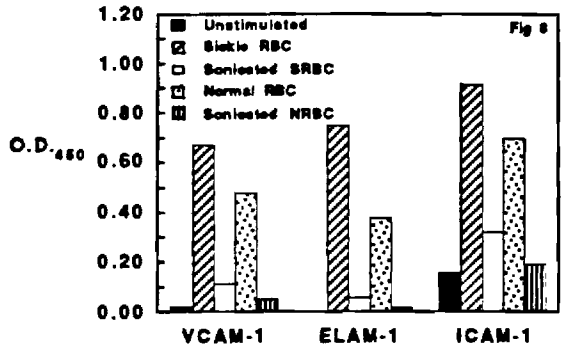

Figure 8. Red cell sonication inhibits sickle red cell induced VCAM-1 expression. Endothelial cells were incubated with sonicated or intact washed sickle or normal red cells for 8 hours and protein expression was determined by ELISA

Figure 9. Sickle erythrocytes are more adherent to endothelial cell monolayers after prolonged stimulation with sickle red cells. Results are from one experiment.

sickle cell
and thus establish a possible link between viral infection and sickle cell adherence
These investigations are the result of collaborations with Project #2. Additional details of the effects of sickle cells are presented in that progress report as well as details related to future investigations. Since project #1 is primarily related to sickle red cell/endothelial cell adhesion, future studies along these lines will be primarily related to the effect of sickle cells on endothelial cell adhesivity.

3. Significance

Double stranded RNA induces sickle cell adherence.

Previous studies suggest that pain events in sickle cell patients may by initiated or exacerbated by infection, however the link between infection and vascular complications in sickle cell anemia is not clear. Many pathogenic viruses, including rhinoviruses, influenza viruses, measles viruses, coxsackie viruses, enteroviruses, and flaviviruses exist as double stranded RNA viruses or replicate through double stranded RNA intermediates (19). Recent studies have demonstrated that double stranded RNA illicits many of the pathogenic complications in animals as the intact virus, suggesting that double stranded RNA may be a reasonable model for viral infection (12). The data of Figures 1-3 indicating that double stranded RNA specifically induces endothelial cell VCAM-1 expression and sickle erythrocyte adherence via a VCAM-1/$\alpha_4\beta_1$ interaction suggests that viral infection in sickle cell disease may initiate microvascular occlusion by promoting sickle red cell adherence to endothelium *in vivo*. Future studies, in collaboration with Dr. Margaret K. Offermann, Department of Hematology/Oncology, School of Medicine, Emory University School of Medicine will focus on the mechanism of double stranded RNA induced VCAM-1 induction and whether intact viruses increase sickle red cell adherence to endothelium (20) in a manner similar to that induced by double stranded RNA.

4. Plans

In general, we do not anticipate significant deviation from the original proposal.

Literature Cited

1. Kaul DK, ME Fabry, RL Nagel. Microvascular sites and characteristics of sickle cell adhesion to vascular endothelium in shear flow conditions: Pathophysiological implications. Proc Natl Acad Sci, USA 86:3356; 1989.
2. Brittain HA, JR Eckman, RJ Howard TM Wick: Thrombospondin from activated platelets promotes sickle erythrocyte adherence to human microvascular endothelium under physiologic flow: A potential role for platelet activation in sickle cell vaso-occlusion. Blood, 81:2137-2143 (1993).
3. Brittain HA: Adherence of Sickle Red Cells to Human Microvascular Endothelial Versus Umbilical Vein Endothelial Cells; A Role for Plasma, von Willebrand Factor, and Platelet Thrombospondin," Ph.D. dissertation, Georgia Institute of Technology, August 1993.
4. Wick TM, JL Moake, MM Udden, SG Eskin, DA Sears, and LV McIntire. Unusually large von willebrand factor multimers increase adhesion of sickle erythrocytes to endothelial cells under controlled flow." Journal of Clinical Investigation, 80:905-910 (1987).
5. Wick, TM, JL Moake, MM Udden, and LV McIntire. Unusually large von willebrand factor multimers preferentially promote young sickle and non-sickle erythrocyte adhesion to endothelial cells," American Journal of Hematology, 42:284-292 (1993).
6. Wick TM, JL Moake, MM Udden, SG Eskin, DA Sears and LV McIntire. Unusually large von willebrand factor multimers increase adhesion of sickle erythrocytes to endothelial cells under controlled flow. J Clin Invest 80:905; 1987.
7. Wick TM, JL Moake, MM Udden, and LV McIntire. Unusually large von willebrand factor multimers preferentially promote young sickle and non-sickle erythrocyte adhesion to endothelial cells," Am J Hematology, 42:284; (1993).
8. Barrett-Connor E. Bacterial Infection and Sickle Cell Anemia: Medicine 50:98; 1971.
9. Wautier JL, F Galacteros, MP Wautier, D Pintigny, Y Beuzard, J Rosa, J Caen. Clinical manifestations and erythrocyte adhesion to endothelium in sickle cell syndrome. Am J Hematol 19:121; 1985.
10. Mendoza E, N Gutgsell, JD Temple, P Issitt. Monocyte phagocytic activity in sickle cell disease. Acta Haematol 85:199; 1991.
11. Swerlick, R.A., J.R. Eckman, A. Kumar, M. Jeitler, and T.M. Wick. $a_4/ß_1$-Integrin Expression on Sickle Reticulocytes: Vascular Cell Adhesion Molecule-1-Dependent Binding to Endothelium Blood 82:1891; 1993.
12. Kimura-Takeuchi M, JA Majde, LA Toth, JM Krueger. The role of double stranded RNA in induction of the acute-phase response in an abortive influenza virus infection model. J Infect Dis 166:1266; 1992.
13. Yang J, MK Hagan, RM Medford, MK Offermann. Vascular endothelial cells express multiple pro-inflammatory genes in response to double stranded RNA. J Immunol 152:1; 1994.
14. Elices MJ, L Osborn, Y Takada, C Crouse, S Luhowskyj, ME Hemler, RR Lobb. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. Cell 60:477, 1990.
15. Swerlick RA, KH Lee, L-J Li, NT Sepp, SW Caughman, TJ Lawley. Regulation of vascular cell adhesion molecule 1 on human dermal microvascular endothelial cells. J Immunol 149:698; 1992.
16. Powars DR, B Wilson, C Imbs, C Pegelow, J Allen. The natural history of stroke in sickle cell disease. Am J Med 65:461; 1978.
17. Russell MO, HI Goldberg, A Hodson, HC Kim, J Halus, M Reivich, E Schwartz. Effect of transfusion therapy on arteriographic abnormalities and on recurrence of stroke in sickle cell disease. Blood 63:162; 1984.

18. Adams RJ, FT Nichols, V McKie, K McKie, P Milner, T El Gammal. Cerebral infarction in sickle cell anemia: mechanisms based on CT and MRI. 38:1012; 1988.
19. Murphy F and D Kingsbury. Virus taxonomy. In: Fields virology, 2nd edition, DM Knipe, RM Chanock, MS Hirsch, JL Melnick TP Monath, B Roizman, eds., Raven Press, New York, pp. 9-36, 1990.
20. Hebbel RP, MR Visser, JL Goodman, HS Jacob, GM Verellotti. Potentiated adherence of sickle erythrocytes to endothelium infected by viruses. J Clin Invest 80:1503, 1987.

5. Human Subjects

a. General Guidelines

i. Proposed Use

Patients with sickle-cell syndromes (HbSS, HbSC, HbS b-thalassemia) not receiving anticoagulant therapy and without evidence of pregnancy, obvious infection, thromboembolic disease or liver disease will be eligible for this study. Patients will be studied once in pain crisis and twice during asymptomatic periods. An age and sex matched population of normal black individuals will serve as a control population. Approximately twenty patients and twenty control subjects, aged eighteen or older, will be studied annually. Ten milliliters of blood will be drawn by venipuncture for each experiment.

ii. Specimen Usage

None of the data from the experiments will be used for diagnosis or treatment of specific individuals.

iii. Patient Recruitment

Patients from the Sickle Cell Center or the in-patient service at Grady Memorial Hospital, Atlanta, GA and hospital staff will be recruited by Dr. James R. Eckman. Subjects will agree to participate in this study by signing a consent form approved by Georgia Tech and Emory University School of Medicine IRBs. The consent form explains the nature of the study, the details of blood collection, risks associated with drawing blood, the availability of personnel to discuss the results of the study, the assurance of anonymity, and the ability to withdraw from the study at any time without penalty or loss of benefits.

iv Potential Risks

The risks of drawing blood are minimal and include slight pain, bruising, and infection at the site of puncture. No viable alternative for drawing human blood exists.

v. Procedures to Minimize Risk

Patient confidentiality will be ensured by assigning a code to each patient studied (SS1, AA1 for sickle and normal donor, respectively) to be used when all data is reported. Blood will be drawn at Grady Hospital under the supervision of Dr. James R. Eckman, director of the Sickle Cell Clinic. Dr. Eckman will be available to answer questions and to arrange for emergency medical care if a medical problem develops during the course of this study.

vi. Justification

The risk of blood drawing is minimal compared to potential benefits of a better understanding of clotting abnormalities in sickle cell syndromes and their relationship to pain crisis.

b. Gender and Minority Inclusions

Study subjects will be patients diagnosed with sickle cell syndromes as defined above. These patients will primarily be of African descent, however no patients will be included or excluded on the basis of race. The study population will consist of approximately equal numbers of men and women.

Exclusion criteria will be solely based on medical criteria as described above. Control subjects (volunteers without hemoglobinopathies) will be age, sex, and race-matched. These volunteers are recruited from the hospital staff at Grady Memorial Hospital in Atlanta.

6. Vertebrate Animals

None.

7. Publications (from this project)

a. Journal Articles

b. Abstracts and Meeting Presentations

7. Inventions and Patents

None.

All Personnel for the Current Budget Period
and Any Planned Changes in Personnel for the Next Budget Period

Use two sections. In the first section list *All Current Personnel*. In the second section list *Planned Personnel Changes.*

Name	Degree(s)	SSN	Role on Project *(e.g., PI, Res. Assoc.)*	Date of Birth *(MM/DD/YY)*	Annual % Effort
Current Personnel					
Timothy M. Wick	B.S.,Ph.D.	505-94-2891	PI	07/09/61	20%
James R. Eckman	B.A.,M.D.	471-48-8946	Co-Investigator	08/25/43	5%
Paula A. Smolinski	B.S.		Graduate Student		100%
Henri A. Brittain	B.S.,M.S., Ph.D.	253-81-0772	Graduate Student	01/18/59	100%
Planned Changes addition:					
Richard Montez	B.S.,M.S.		Graduate Student		100%
Mr. Montez will replace Mr. Brittain, who graduated.					
Marena Brown	B.S., M.S.		Graduate Student		100%
Ms. Brown is responsible for the endothelial cell stimulation studies.					

Provide the number of subjects enrolled in the study *to date* according to the following categories. (See Page 8 for definitions.)

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown	TOTAL
Female							
Male							
Unknown							
TOTAL							

CHECKLIST

GRANT NUMBER

1P60 HL48482-02

Check the appropriate boxes and provide the information requested. Make this page the last page of the signed original of the application.

1. ASSURANCES/CERTIFICATIONS

The following assurances/certifications are made by checking the appropriate boxes and verified by the signature of the OFFICIAL SIGNING FOR APPLICANT ORGANIZATION on the FACE PAGE of the application. Descriptions of individual assurances/certifications begin on page 9 of Specific Instructions.

a. Human Subjects *(Complete Item 7 on the Face Page)*
- [x] Full IRB Review [ ] Expedited Review
- Use of Human Subjects [ ] Change [x] No Change Since Previous Submission

b. Vertebrate Animals *(Complete Item 8 on the Face Page)*
- Use of Vertebrate Animals [ ] Change [ ] No Change Since Previous Submission

c. Inventions and Patents *(Complete Item 11 on the Face Page)* None.

d. Debarment and Suspension [x] No [ ] Yes *(Attach explanation)*

e. Lobbying
- With Federal appropriated funds [x] No
- With other than Federal appropriated funds [ ] No [ ] Yes
- (If "yes", see page 13, and attach Standard Form LLL, "Disclosure of Lobbying Activities," to the application behind the Checklist.)

f. Delinquent Federal Debt [x] No [ ] Yes *(Attach explanation)*

g. Misconduct in Science (Form PHS 6315) [x] Filed [ ] Not Filed
- If filed, date of Initial Assurance or latest Annual Report 2/25/93

h. Civil Rights	i. Handicapped Individuals	j. Sex Discrimination	k. Age Discrimination
Form HHS 441	Form HHS 641	Form HHS 639-A	Form HHS 680
[x] Filed	[x] Filed	[x] Filed	[x] Filed
[ ] Not Filed	[ ] Not Filed	[ ] Not Filed	[ ] Not Filed

2. PROGRAM INCOME *(See Instructions, Page 16)*

All applications must indicate (Yes or No) whether program income is anticipated during the period(s) for which grant support is requested. [x] No [ ] Yes. If "Yes" use the format below to reflect the amount and source(s) of anticipated program income.

Budget Period	Anticipated Amount	Source(s)

3. INDIRECT COST

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office, or, in the case of forprofit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office. Indirect costs will not be paid on foreign grants, construction grants, grants to Federal organizations and grants to individuals, and usually not on conference grants. Follow any additional instructions provided for Research Career Development Awards, Institutional National Research Service Awards, and specialized grant applications.

- [ ] DHHS Agreement Dated: _____ [ ] No Indirect Costs Requested
- [x] No DHHS Agreement, but rates established with Office of Naval Research Date 29 June 1993

CALCULATION*

Enter proposed budget period:

Amount of base $80,144 × Rate applied 37 % = Indirect costs $ 29,653

Add to total direct costs from form page 2 and enter new total on Face Page, item 10b

*Check appropriate box(es):
- [ ] Salary and wage base [x] Modified total direct costs base [ ] Other base (Attach explanation)
- [ ] Off-site, other special rate, or more than one rate involved *(Attach explanation)*

Form approved through 6/30/94
OMB No. 0925-0001

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

APPLICATION FOR CONTINUATION GRANT

REVIEW GROUP	TYPE	ACTIVITY	GRANT NUMBER
			1P60 HL48482-03

TOTAL PROJECT PERIOD
From: 1 April 1993 Through: 31 March 1998

REQUESTED BUDGET PERIOD
From: 1 April 1995 Through: 31 March 1996

1. TITLE OF PROJECT
Georgia Comprehensive Sickle Cell Center - Project #1

2a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR *(Name and address, street, city, state, zip code)*

Timothy M. Wick, Ph.D.
School of Chemical Engineering
Georgia Institute of Technology
778 Atlantic Drive
Atlanta, GA 30332-0100

BITNET/INTERNET ADDRESS
timothy.wick@che.gatech.edu

2b. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT
School of Chemical Engineering

2c. MAJOR SUBDIVISION
College of Engineering

3. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR BIOMEDICAL RESEARCH SUPPORT GRANT *(See instructions)*

20 Other

4. APPLICANT ORGANIZATION *(Name and address, street, city, state, zip code)*

Georgia Tech Research Corporation
OCA/PID Room 246 CRB
Georgia Institute of Technology
Atlanta, GA 30332-0420

5. ENTITY IDENTIFICATION NUMBER
1580603146A1

6. TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL

Office of Contract Administration
Georgia Institute of Technology
Atlanta, GA 30332-0420

BITNET/INTERNET ADDRESS

7. HUMAN SUBJECTS — If "YES" exemption no. or IRB approval date: M1395 — 4b. Assurance of compliance no.
[ ] 7a. NO [X] YES

8. VERTEBRATE ANIMALS — If "YES," IACUC approval date — 8b. Animal welfare assurance no.
[X] 8a. NO [ ] YES

9. PERFORMANCE SITE(S) *(Organizations and addresses)*

Georgia Institute of Technology
Cellular Adhesion Laboratory
Space Science and Technology-Building A
Room 217
275 Ferst Drive
Atlanta, GA 30332-0405

10. COSTS REQUESTED FOR NEXT BUDGET PERIOD
10a. DIRECT $ 86,439 10b. TOTAL $ 121,015

11. INVENTIONS AND PATENTS *(See instructions)*
[X] NO [ ] YES If "YES," [ ] Previously reported [ ] Not previously reported

TELEPHONE AND FAX INFORMATION

	AREA CODE	TELEPHONE NO. AND FAX NO.
12a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR *(Item 2a)* Timothy M. Wick	404	894-8795
	404	~~894-2866 (fax)~~
12b. NAME OF ADMINISTRATIVE OFFICIAL *(Item 6)* Christopher E. D'Urbano	404	894-4817
	404	894-6956 (fax)
12c. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION *(Item 15)* Christopher E. D'Urbano Contracting Officer		

BITNET/INTERNET ADDRESS david.bridges@oca.gatech.ed

13. DO NOT USE THIS SPACE

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application. Willful provision of false information is a criminal offense (U.S. Code, Title 18, Section 1001). I am aware that any false, fictitious, or fraudulent statement may, in addition to other remedies available to the Government, subject me to civil penalties under the Program Fraud Civil Remedies Act of 1986 (45 CFR 79).	SIGNATURE OF PERSON NAMED IN 2a *(In ink. "Per" signature not acceptable.)*	DATE 12/12/94
15. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with the Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense (U.S. Code, Title 18, Section 1001). I am aware that any false, fictitious, or fraudulent statement may, in addition to other remedies available to the Government, subject me to civil penalties under the Program Fraud Civil Remedies Act of 1986 (45 CFR 79).	SIGNATURE OF PERSON NAMED IN 12c *(In ink. "Per" signature not acceptable.)*	DATE 12/14/94

DETAILED BUDGET FOR NEXT BUDGET PERIOD DIRECT COSTS ONLY	FROM	THROUGH	GRANT NUMBER
	1 April 1995	31 March 1996	1P60 HL48482-03

PERSONNEL (Applicant organization only) NAME	ROLE ON PROJECT	TYPE APPT. (months)	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED (Omit cents) SALARY REQUESTED	FRINGE BENEFITS	TOTALS
Timothy M. Wick, Ph.D.	PI	12	20	$75,484	$15,097	$3,729	$18,826
James R. Eckman	CO-PI	12	5				
Paula A. Smolinski	Graduate Student	12	100	$15,000	$15,000	-	$15,000
Richard Montes	Graduate Student	12	100	$15,000	$15,000	-	$15,000
To be Named	Cell Culture Technician	12	40	$32,375	$12,950	$3,199	$16,149
SUBTOTALS →					$58,047	$6,928	$64,975

CONSULTANT COSTS

NONE

EQUIPMENT *(Itemize)*

NONE

SUPPLIES *(Itemize by category)*

Tissue culture supplies (medium, serum, growth factors, etc.) attachment factors	$6,864
Monoclonal antibodies, synthetic pestides	$5,000
Disposable supplies (culture flasks, pipets, fillters, gloves, etc.)	$4,160
Elisa Reagents, FACS Supplies	$1,418
	$17,442

TRAVEL

Travel to two scientific meetings for investigators — $ 1,622

PATIENT CARE COSTS

INPATIENT NONE

OUTPATIENT NONE

ALTERATIONS AND RENOVATIONS *(Itemize by category)*

NONE

OTHER EXPENSES *(Itemize by category)*

Publication fees, artwork, photography	$1,500
Shop charges for flow loop manufacture	$ 900
	$ 2,400

SUBTOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD

~~CONSORTIUM/CONTRACTUAL COSTS~~

DIRECT COSTS $ 86,439

INDIRECT COSTS $ 34,576 (40% of Direct)

TOTAL →

TOTAL DIRECT COSTS FOR NEXT BUDGET PERIOD *(Enter on Page 1, Item 10a)* → $ Direct $ 86,439 Total $121,015

BUDGET JUSTIFICATION	GRANT NUMBER
	1P60 HL48482-03

SUPPLEMENTAL INFORMATION REGARDING *ITEMS* IN THE PROPOSED BUDGET FOR THE NEXT PERIOD WHICH REQUIRE EXPLANATION OR JUSTIFICATION. *(See instructions)*

Personnel: Fringe benefits are 24.7% of salary.

Principal Investigator - Dr. Timothy M. Wick, Ph.D.: Funding is requested for the Principal Investigator to provide time to organize the study, coordinate *in vitro* investigations with clinical studies, perform experiments, analyze data, prepare manuscripts, hold regular laboratory meetings of the investigators, and develop progress reports. It is estimated that 20% of Dr. Wick's time will be devoted to these tasks related to the Project #1 of the Georgia Comprehensive Sickle Cell Center.

Graduate Student - Paula Smolinski: Ms. Smolinski has been working in the laboratory since September 1992. She is responsible for much of the new data presented in the Results section. Specifically, Ms. Smolinski has performed the investigations demonstrating that infection of endothelial cells with double stranded RNA or a double stranded RNA virus increases sickle cell adherence via $\alpha_4\beta_1$/VCAM-1. She has also performed the detachment assays and will continue to be responsible for measuring the strength of sickle cell adherence via the various pathways. Ms. Smolinski will devote 100% of her effort to this project.

Graduate Student - Richard Montez: Mr. Montez has been working in the lab since December 1993. He has developed the cone-and-plate viscometer shown in the Progress Report and the protocols for measuring the kinetics of sickle cell adherence described in the present Continuation Application. Mr. Montez will devote 100% of his effort to this project.

Graduate Student - Marena Brown: Ms. Brown has been working in the lab since Summer of 1992. Ms. Brown is investigating the effect of plasma components in sickle blood (such as leukocytes and cytokines) on the activation of endothelial cells *in vivo*. She is currently working to identify the specific component(s) in sickle blood that induce endothelial cell expression of cell adhesion molecules. Ms. Brown is currently supported by other funds and no support is being requested for her from this application.

Cell Culture Technician: This person is responsible for maintaining endothelial cell cultures. This entails harvesting cells from tissues or procuring from other sources; maintaining viable cell stocks; and providing cells for use in adherence assays and ELISAs. This person is required because we are using endothelial cells from different sources (e.g. veins, arteries, and microvessels) each of which has exacting and different culture requirements. Having one person as an expert in endothelial cell culture is invaluable to efficient and reproducible progress on this and other related projects.

CURRENT BUDGET PERIOD	FROM	THROUGH
	1 April 1995	31 March 1996

The following pertains to your CURRENT PHS budget. This information may be used in determining the amount of support for the NEXT budget period.

A. CURRENT BUDGET	TOTAL ESTIMATED EXPENDITURES AND OBLIGATIONS (1)	ESTIMATED UNOBLIGATED BALANCE (2)	EXPLAIN ANY SIGNIFICANT ESTIMATED UNOBLIGATED BALANCE IN COLUMN 2 (3)
TOTAL DIRECT COSTS	$ 73,996	0	
INDIRECT COSTS *(As provided)*	$ 29,440	0	
TOTALS →	$103,592	0	

Supplies: Tissue culture costs are based upon current performance of 2 flow experiments per week as well as current usage and costs. Media, serum, growth factors, buffers, and other chemicals as well as plasticware, glassware, and gloves are required for cell cultures and adhesion assays. Monoclonal antibodies to adhesion receptors will be used to identify receptors that are necessary for sickle erythrocyte adhesion to endothelium.

Travel: Funds are requested for Dr. Wick or an associate to attend ASH and the annual Meeting of the Sickle Cell Disease Program to present research and interact with colleagues interested in similar and related areas of hematology and sickle cell anemia.

Other Expenses: Funds are requested to cover the cost photocopying, medical illustrations and page costs for presentations and publications. Machine shop charges are required to construct new adhesion systems.

BIOGRAPHICAL SKETCH

Give the following information for all new key personnel, consultants, and collaborators.
Copy this page for each person.

NAME	POSITION TITLE
Timothy M. Wick	Associate Professor

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Colorado, Boulder, CO	B.S.	1983	Chemical Engineering
Rice University, Houston, TX	Ph.D.	1988	Chemical Engineering
Rice University, Houston, TX	Post-doc	1988	Chemical Engineering and Biochemistry

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individual who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience

7/94-present Associate Professor, School of Chemical Engineering, Georgia Institute of Technology, Atlanta, GA

7/94-present Associate Professor, School of Mechanical Engineering, Georgia Tech, Atlanta, GA

4/93-6/94 Assistant Professor, School of Mechanical Engineering, Georgia Tech, Atlanta, GA

9/88-6/94 Assistant Professor, School of Chemical Engineering, Georgia Institute of Technology, Atlanta, GA

2/88-9/88 Post-doctoral Research Associate, Department of Chemical Engineering, Rice University, Houston, TX

2/88-9/88 Post-doctoral Research Associate, Department of Chemical Engineering, Rice University, Houston, TX

Honors and Awards

1993 Ad-Hoc Reviewer. Academic Research Initiation Grants Program, North Carolina Biotechnology Center.

1993 Ad-Hoc Reviewer. Biomaterials Panel, Biomedical Engineering and Research to Aid Persons with Disabilities Program, National Science Foundation .

1993 Member. Special Emphasis Panel, NIH Collaborative Projects (RO1s) on Minority Health.

1993 Reviewer. Biomedical Engineering Society 1993 Graduate Student Awards Committee.

1993 Outstanding Chemical Engineering Professor. Awarded by the Student Chapter of Omega Chi Epsilon.

1992 Lilly Foundation Teaching Fellowship.

1991 Young Investigator Award Finalist. The 1991 World Congress on Medical Physics and Biomedical Engineering.

1991 American Heart Association-Georgia Affiliate, Grant-In-Aid

1991 The Whitaker Foundation, Biomedical Engineering Research Grant

1991 NIH-First Independent Research and Transition (FIRST) Award

1990 Du Pont Young Faculty Award

1991 Du Pont Young Faculty Award

1989 American Heart Association-Georgia Affiliate, Grant-In-Aid

1987 Beecham Award for outstanding original research presented at annual meeting of the Southern Society for Clinical Investigation, the Southern Section of the AFCR and the Southern Society for Pediatric Research

1986 Omega Chi Epsilon (National Chemical Engineering Honor Society)

Original Articles

1. Wick, T.M., J.L. Moake, M.M. Udden, S.G. Eskin, D.A. Sears and L.V. McIntire. "Unusually Large von Willebrand Factor Multimers Increase Adhesion of Sickle Erythrocytes to Endothelial Cells Under Controlled Flow." Journal of Clinical Investigation, **80**:905-910 (1987).
2. Wick, T.M., S.D. Doty, and R.M. Nerem. "Influence of Fluid Mechanical Stresses on Vascular Cell Adhesion." In: Biomechanical Transport Processes, F. Mosora, C. Caro, E. Krause, H. Schmid-Schönbein, C. Baquey, and R. Pelissier, eds, Plenum, New York, pp. 283-292, 1990.
3. Wick, T.M. and V. Louis. "Cytoadherence of *Plasmodium falciparum*-Infected Erythrocytes to Human Umbilical Vein and Human Dermal Microvascular Endothelial Cells under Shear Conditions." Am J Tropical Med Hygiene **45**: 578-586 (1991).
4. Swerlick, R.A., K. Lee, T.M. Wick, and T.J. Lawley. "Human Dermal Microvascular Endothelial but not Human Umbilical Vein Endothelial Cells Express CD36 *In Vivo* and *In Vitro*." Journal of Immunology, **148**:78-83 (1992).
5. Yoganathan A.P., T.M. Wick, & H. Reul. "The Influence of Flow Characteristics of Prosthetic Valves on Thrombus Formation." In: Thrombosis, Embolism, and Bleeding, E.G. Butchart and E. Bodnar, eds, ICR Publishers, London, pp. 123-148, 1992.
6. Wick, T.M. and V. Louis. "*Plasmodium fragile*: Cytoadherence of Parasitized Rhesus Monkey Erythrocytes to Human Endothelial Cells under Shear Flow Conditions." Experimental Parasitology, **74**:228-231 (1992).
7. Brittain, H.A., J.R. Eckman, and T.M. Wick. "Sickle Erythrocyte Adherence to Large Vessel and Microvascular Endothelium under Physiologic Flow is Qualitatively Different." J Lab Clin Med, **19**:538-545 (1992).
8. Wick, T.M., J.L. Moake, M.M. Udden, and L.V. McIntire. "Unusually Large von Willebrand Factor Multimers Preferentially Promote Young Sickle and Non-sickle Erythrocyte Adhesion to Endothelial Cells," Am J Hematology, **42**:284-292 (1993).
9. Johnson, J.K., R.A. Swerlick, P. Millet, K. Grady, T.M. Wick. "Cytoadherence of *Plasmodium falciparum*-Infected Erythrocytes to Microvascular Endothelium is Regulatable by Cytokines and Phorbol Ester," J Infect Dis, **167**:698-703 (1993).
10. Brittain, H.A., J.R. Eckman, R.J. Howard, and T.M. Wick. "Thrombospondin from Activated Platelets Promotes Sickle Erythrocyte Adherence to Human Microvascular Endothelium under Physiologic Flow: A Potential Role for Platelet Activation in Sickle Cell Vaso-occlusion," Blood, **81**:2137-2143 (1993).
11. Flaherty, A.L. and T.M. Wick. "Prolonged Contact with Blood Alters Surgical Gown Permeability," The American Journal of Infection Control, **21**:249-256 1993.
12. Swerlick, R.A., J.R. Eckman, A. Kumar, M. Jeitler, and T.M. Wick. "$\alpha_4\beta_1$-Integrin Expression on Sickle Reticulocytes: Vascular Cell Adhesion Molecule-1-Dependent Binding to Endothelium," Blood, **82**:1891-99 (1993).
13. Wick, T.M., H.A. Brittain, R. Howard, and J.R. Eckman. "Thrombospondin from Activated Platelets Promotes Sickle Erythrocyte Adherence to Human Microvascular Endothelial Cells via CD36 and integrin receptors," In: Vascular Endothelium: Physiological Basis of Clinical Problems II, J. Catravas, A. Callow, N. Gillis, U. Ryan, A. Mantovani, and M. Yacoub, eds, Plenum, New York, (**In press**) 1994.
14. Smolinski, P.A., M.K. Offermann, J.R. Eckman, and T.M. Wick. "Synthetic Double Stranded RNA Increases Sickle Red Blood Cell Adherence to Endothelium via a VCAM-1/VLA-4 Pathway," Blood (In review).
15. Gonzales, R. and T.M. Wick. "The Dual Effect of Shear Stress in Regulating Monocyte Adherence to Vascular Endothelium," Annals of Biomedical Engineering (In review).
16. Kumar, A., R.A. Swerlick, J.R. Eckman, and T.M. Wick. "Stimulation of Sickle Erythrocytes with Phorbol Ester Promotes Endothelial Adherence: A Novel Adherence Pathway involving Activated $\alpha_4\beta_1$ and Fibronectin," Blood (In preparation).

OTHER SUPPORT *(Use continuation pages if necessary)*	GRANT NUMBER 1 P60 HL48482-03

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to significant delays in the review and/or funding of the application.

Other support is defined as all funds or resources, whether Federal, non-Federal, or institutional, available to the principal investigator/program director (and other key personnel named in the application) in direct support of their research endeavors through research or training grants, cooperative agreements, contracts, fellowships, gifts, prizes, and other means.

Reporting requirements are: For each of the key personnel, describe (1) all currently *active* support and (2) all applications and proposals *pending* review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check "None." Use continuation pages as needed to provide the required information in the *format* as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

Name Timothy M. Wick Active X Pending _____ None _____

a. Source and identifying no. NIH 1R29 HL44960 P.I. T. M. Wick

Title The Mechanism of Sickle Erthrocyte/Endothelial Adherence

b. Your role on project Principal Investigator % Effort 50%

c. Dates and costs of entire project 25 July 1991 - 30 June 1996 $349,992 (direct)

d. Dates and costs of current year 1 July 1994 - 30 June 1995 ($70,539)

e. Specific aims of project (1) Characterize sickle erythrocyte adhesion to endothelium from different vascular beds, (2) determine inter- and intra-patient variability in adhesion for patients in steady state and crisis, (3) identify plasma factors which promote adherence, (4) identify the role of agonists and vaso-active factors in adherence. The R29 research involves different graduate students and although complementary, is separate from the SCORE research.

f. Describe scientific and budgetary overlap None

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

None

OTHER SUPPORT *(Use continuation pages if necessary)*	GRANT NUMBER 1 P60 HL48482-03

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Reporting requirements are: For each of the key personnel, describe (1) all currently *active* support and (2) all applications and proposals *pending* review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check "None." Use continuation pages as needed to provide the required information in the *format* as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

Name Timothy M. Wick, Ph.D **Active** X **Pending** _____ **None** _____

a. Source and identifying no. NIH 1P60 HL48482-01 Project 2 **P.I.** J.R. Eckman

Title Georgia Comprehensive Sickle Cell Center-Project #2

b. Your role on project Collaborating Investigator on Project 2 **% Effort** 10%

c. Dates and costs of entire project 1 April 1993 - 31 March 1998 $597,671 (total project 2)

d. Dates and costs of current year 1 April 1994 - 31 March 1998 $112,655

e. Specific aims of project This project elucidates the effects of sickle erythrocytes on endothelial cell structure and function. These studies are aimed at elucidating the role of sickle erythrocyte membranes in sickle cell vascular pathology.

Dr. Wick draws 10% salary from this project and contributes 10% effort.

f. Describe scientific and budgetary overlap None

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

None

OTHER SUPPORT

(Use continuation pages if necessary)

GRANT NUMBER

1 P60 HL48482-03

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to significant delays in the review and/or funding of the application.

Other support is defined as all funds or resources, whether Federal, non-Federal, or institutional, available to the principal investigator/program director (and other key personnel named in the application) in direct support of their research endeavors through research or training grants, cooperative agreements, contracts, fellowships, gifts, prizes, and other means.

Reporting requirements are: For each of the key personnel, describe (1) all currently *active* support and (2) all applications and proposals *pending* review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check "None." Use continuation pages as needed to provide the required information in the *format* as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

Name Timothy M. Wick Active X Pending _____ None _____

a. Source and identifying no. Allelix Biopharmaceuticals, Inc. P.I. T. Wick

Title Inhibition of Sickle Cell Adherence by CSVTCG Peptide

b. Your role on project Principal Investigator % Effort 5%

c. Dates and costs of entire project 1 September 1994 - 28 February 1995 $60,065

d. Dates and costs of current year 1 September 1994 - 28 February 1995 $60,065

e. Specific aims of project To determine whether the hexapeptide CSVTCG inhibits thrombospondin-mediated sickle cell adherence to microvascular endothelium.

f. Describe scientific and budgetary overlap None

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

None

OTHER SUPPORT *(Use continuation pages if necessary)*	GRANT NUMBER 1 P60 HL48482-03

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to significant delays in the review and/or funding of the application.

Other support is defined as all funds or resources, whether Federal, non-Federal, or institutional, available to the principal investigator/program director (and other key personnel named in the application) in direct support of their research endeavors through research or training grants, cooperative agreements, contracts, fellowships, gifts, prizes, and other means.

Reporting requirements are: For each of the key personnel, describe (1) all currently *active* support and (2) all applications and proposals *pending* review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check "None." Use continuation pages as needed to provide the required information in the *format* as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

Name Timothy M. Wick, Ph.D. Active X Pending _____ None _____

a. Source and identifying no. The Whitaker Foundation P.I. R. Nerem

Title Biomedical Engineering Education: An interdisciplinary Tissue Engineering Education and Research Program

b. Your role on project Participating Faculty % Effort 0%

c. Dates and costs of entire project 1 September 1993 - 31 August 1996 ($3,000,000)

d. Dates and costs of current year 1 September 1994 - 31 August 1995 ($1,000,000)

e. Specific aims of project Renovate laboratory space, hire new faculty and support a limited number of graduate students in tissue engineering

f. Describe scientific and budgetary overlap None

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

None

OTHER SUPPORT *(Use continuation pages if necessary)*	GRANT NUMBER 1 P60 HL48482-03

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to significant delays in the review and/or funding of the application.

Other support is defined as all funds or resources, whether Federal, non-Federal, or institutional, available to the principal investigator/program director (and other key personnel named in the application) in direct support of their research endeavors through research or training grants, cooperative agreements, contracts, fellowships, gifts, prizes, and other means.

Reporting requirements are: For each of the key personnel, describe (1) all currently *active* support and (2) all applications and proposals *pending* review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check "None." Use continuation pages as needed to provide the required information in the *format* as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

Name Timothy M. Wick, Ph.D. Active X Pending _____ None _____

a. Source and identifying no. NIH NRSA NIGMS GM08433 P.I. R. M. Nerem

Title Cellular Engineering Training Grant

b. Your role on project Collaborating Faculty % Effort 0%

c. Dates and costs of entire project 26 September 1994 - 31 August 1995 ($339,208)

d. Dates and costs of current year 1 September 1994 - 31 August 1995 ($87,787)

e. Specific aims of project This project provides funds for four predoctoral students studying Cellular Engineering. Dr. Wick supervises one of these students who works on sickle cell/endothelial interactions.

f. Describe scientific and budgetary overlap None

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

None

OTHER SUPPORT *(Use continuation pages if necessary)*	GRANT NUMBER 1 P60 HL 48482-03

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to significant delays in the review and/or funding of the application.

Other support is defined as all funds or resources, whether Federal, non-Federal, or institutional, available to the principal investigator/program director (and other key personnel named in the application) in direct support of their research endeavors through research or training grants, cooperative agreements, contracts, fellowships, gifts, prizes, and other means.

Reporting requirements are: For each of the key personnel, describe (1) all currently *active* support and (2) all applications and proposals *pending* review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check "None." Use continuation pages as needed to provide the required information in the *format* as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

Name Timothy M. Wick, Ph.D. Active _____ Pending X None _____

a. Source and identifying no. Biogen, Inc. P.I. T. M. Wick

Title Blocade of Sickle Red Cell Binding by Anti-alpha4 Antibodies

b. Your role on project Principal Investigator % Effort 0%

c. Dates and costs of entire project 1 March 1995 - 28 February 1996 ($25,000 total)

d. Dates and costs of current year 1 March 1995 - 28 February 1996 ($25,000 total)

e. Specific aims of project To set up a micropipet assay system to measure the strength of blood cell adhesion to endothelium under various conditions. This is an equipment grant to provide hardware (pipet manipulators, microscope, video equipment) to set up the assay system.

f. Describe scientific and budgetary overlap None

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

None

OTHER SUPPORT *(Use continuation pages if necessary)*	GRANT NUMBER 1P60 HL48482-02

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to significant delays in the review and/or funding of the application.

Other support is defined as all funds or resources, whether Federal, non-Federal, or institutional, available to the principal investigator/program director (and other key personnel named in the application) in direct support of their research endeavors through research or training grants, cooperative agreements, contracts, fellowships, gifts, prizes, and other means.

Reporting requirements are: For each of the key personnel, describe (1) all currently *active* support and (2) all applications and proposals *pending* review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check "None." Use continuation pages as needed to provide the required information in the *format* as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

Name Timothy M. Wick, Ph.D. Active _____ Pending X None _____

a. Source and identifying no. National Institutes of Health P.I. P. A. Lane

Title Free Fatty Acids in Sickle Pulmonary Vascular Injury

b. Your role on project Collaborator % Effort 5%

c. Dates and costs of entire project $876,937

d. Dates and costs of current year $287,371 (First year)

e. Specific aims of project Free fatty acids cause pulmonary vascular injury by 1) increasing endothelial permeability leading to edema; 2) enhancing endothelial production of vaso-active substances; and 3) increasing adherence of sickle cells, platelets and neutrophils to endothelium. Dr. Wick will contribute to specific aim #3.

f. Describe scientific and budgetary overlap None

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

None

PROGRESS REPORT SUMMARY	GRANT NUMBER 1P60 HL48482	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR James R. Eckman, M.D.	PERIOD COVERED BY THIS REPORT FROM	THROUGH
APPLICANT ORGANIZATION Emory University School of Medicine	1 April 1994	31 March 1995

TITLE OF PROJECT *(Repeat title shown in item 1 on first page)*
Georgia Comprehensive Sickle Cell Center - Project #1
(SEE INSTRUCTIONS)

1. Specific Aims

The tendency for hemoglobin SS to polymerize at low oxygen tension is assumed to be the dominant factor in sickle cell pathology. Since morphological sickling is delayed after hemoglobin deoxygenation, factors which delay red cell microcirculatory transit are likely antecedents to microvascular occlusion, ischemic tissue damage, and pain episodes characteristic of sickle cell anemia. Our central hypothesis is that sickle erythrocyte adherence to microvascular endothelium delays erythrocyte microcirculatory transit. This partial obstruction allows for intracapillary red cell sickling leading to complete occlusion (1). Adherence may be affected by secondary perturbations in hematological parameters such as infection which can activate the endothelium, the red blood cells, or alter blood hemodynamics such that adherence is favored. Our data clearly indicate that plasma, red cell and endothelial cell factors as well as local hemodynamics all likely contribute to adherence and occlusion *in vivo* (2-8). Our **specific aims for this project** are to (i) demonstrate that infection induces endothelial alterations which increase sickle erythrocyte adherence to endothelium, (ii) identify the dominant adherence mechanism(s) in the plasma milieu, and (iii) develop novel adhesion systems to study the interrelationship between adherence, endothelial cell function, and fluid mechanics under conditions which more closely mimic those *in vivo*.

It is becoming clear that red cells, white cells and the fluid mechanical environment interact with the endothelium in such a way as to alter cellular function. These alterations are exacerbated in sickle cell anemia because of the presence of sickle erythrocytes which induce a myriad of alterations in blood. The long-term goal of this research, in close collaboration with Projects 2,3, and 4 is to elucidate the role of not only red cells, endothelium, and hemodynamics in vascular complications associated with sickle cell anemia, but also to determine the extent, if any, to which the thrombolytic and immune systems participate in sickle cell vascular complications.

2. Studies and Results

Elucidate the role of viral infection in sickle cell adherence to endothelium

We recently reported that sickle reticulocytes express the $\alpha_4\beta_1$ integrin complex and can bind to VCAM-1 on cytokine activated endothelium (6). Pain episodes are often associated with febrile events precipitated by viral or bacterial infection (9). Many different cell types, including endothelial, have the capacity to recognize and respond to double stranded RNA (10-12). Recent studies demonstrate that double stranded RNA (DS-RNA) induces cellular adhesion molecules, including VCAM-1, in endothelial cells (13).

We hypothesized that DS-RNA or DS-RNA viruses induce endothelial VCAM-1 expression, leading to sickle cell adherence via the $\alpha_4\beta_1$/VCAM-1 pathway. Last year we presented data that the synthetic double stranded RNA poly(I:C), induces endothelial VCAM-1 expression and that sickle erythrocytes adhere to poly(I:C) stimulated endothelium via $\alpha_4\beta_1$/VCAM-1 (7). Subsequent experiments have demonstrated that an intact double stranded RNA virus, parainfluenza 1 virus (Sendai), can also induce endothelial cell VCAM-1 expression leading to sickle cell adherence via $\alpha_4\beta_1$/VCAM-1 (Figure 1) similar to that induced by poly(I:C).

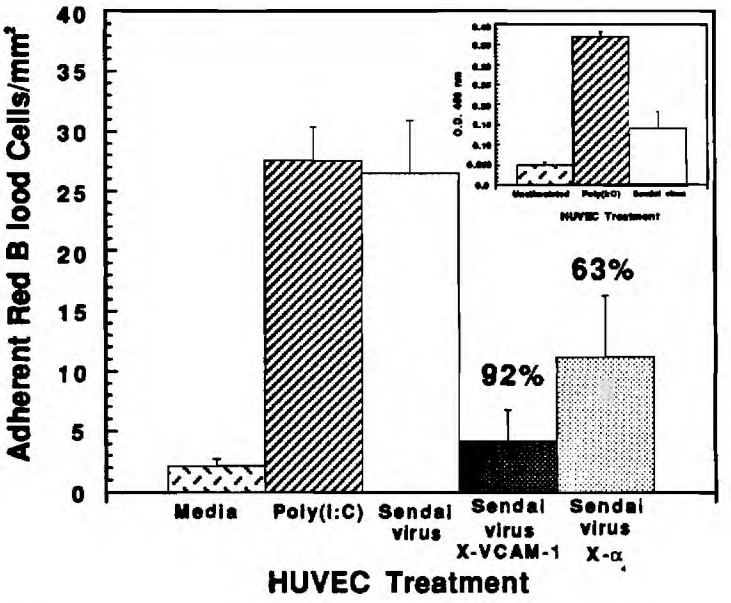

Figure 1. Adherence of sickle erythrocytes to endothelium infected with DS-RNA virus occurs via an $\alpha_4\beta_1$/VCAM-1 mediated mechanism. Where indicated, endothelial cells were incubated with anti-VCAM-1 antibody (X-VCAM-1) or erythrocytes were incubated with anti-α_4 antibody (X-α_4). Data are mean±SEM. Percent antibody blocking is indicated above the bars. Inset: Endothelial VCAM-1 expression was quantified by ELISA after a 1 hr treatment of EC with Sendai virus and a 19 hr post-infection culture period (right bar), a 20 hour EC treatment with poly(I:C) (middle bar), or no EC treatment (left bar)..

Biophysical characterization of adherence pathways

In addition to $\alpha_4\beta_1$/VCAM-1, sickle cells adhere to endothelium via at least three other receptor-mediated pathways: (i) unusually large von Willebrand factor multimers bridging integrin and GPIb-like receptors on sickle cells and endothelium (2,3); (ii) thrombospondin-mediated adhesion via CD36 on sickle cells and CD36 or $\alpha_v\beta_3$ on microvascular endothelium (5) or $\alpha_v\beta_3$ on umbilical vein endothelium (14), and (iii) phorbol ester activated $\alpha_4\beta_1$ on sickle cells binding to fibronectin on endothelium (8).

Thus, sickle erythrocyte adherence to endothelium *in vivo* is likely complex and can occur via multiple and different adherence pathways.

In order to determine the conditions under which these pathways may dominate *in vivo*, we have initiated biophysical studies to compare the strength of sickle cell adherence via different pathways. In these studies, sickle cells are attached to endothelium under static conditions or at a shear stress of 0.5 or 1.0 dyne/cm^2 under conditions where only one adherence pathway is possible. Then, the hemodynamic shear stress is increased sequentially by 0.5 dyne/cm^2 increments. At each shear stress the number of adherent cells remaining is quantified.

Even thought the initial attachment via the $\alpha_4\beta_1$/VCAM-1 and thrombospondin pathways were essentially equivalent at an attachment shear stress of 1.0 dyne/cm^2, more sickle cells remained adherent at every shear stress for the $\alpha_4\beta_1$/VCAM-1 pathway as compared to the thrombospondin pathway (Figure 2). This is especially evident when the data are normalized to the percentage of the cells initially attached at 1 dyne/cm^2 which remain adherent at each higher shear stress (Figure 3).

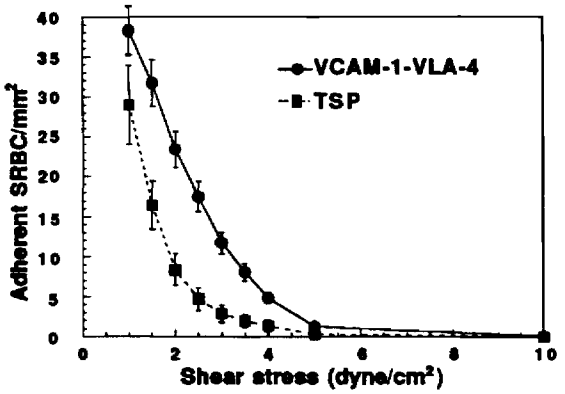

Figure 2. Sickle cell adherence as a function of wash-out shear stress. Sickle red cells were attached to the endothelium under a shear stress of 1.0 dyne/cm^2 and washed off at sequentially increasing shear stress.

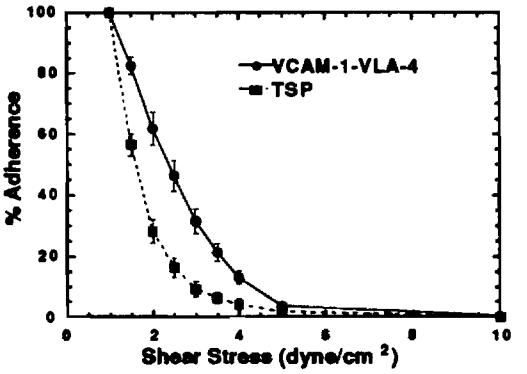

Figure 3. Percentage of initially attached sickle cells remaining adherent as a function of wash-out shear stress.

The conditions of sickle cell attachment also effect the number of sickle cells remaining adherent at subsequently higher shear stresses (Figures 4&5). In these experiments, sickle cells were flowed over endothelial cell monolayers stimulated with poly(I:C) (to induce VCAM-1 expression) at 0.5 or 1.0 dyne/cm^2 shear stress or statically incubated with stimulated endothelium. Then, shear stress was increased in 0.5 dyne/cm^2 increments and sickle cell adherence was quantified. Initial sickle cell attachment was greatest at 0.5 dyne/cm^2 (Figure 4). However, as the wash-out shear stress was increased, the sickle cells adhered under 1 dyne/cm^2 shear stress adhered most tenaciously (Figures 4&5).

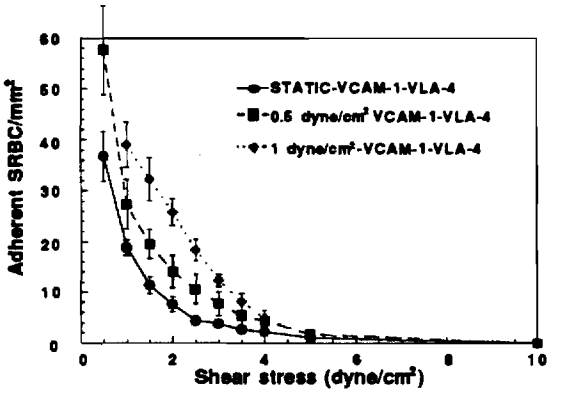

Figure 4. Effect of attachment shear stress on the tenacity of sickle cell adherence to endothelium via $\alpha_4\beta_1$/VCAM-1. The three curves represent the number of red cells remaining adherent to endothelium at the indicated shear stress for sickle cells initially attached to the endothelium during a static incubation or at a shear stress of 0.5 or 1.0 dyne/cm^2.

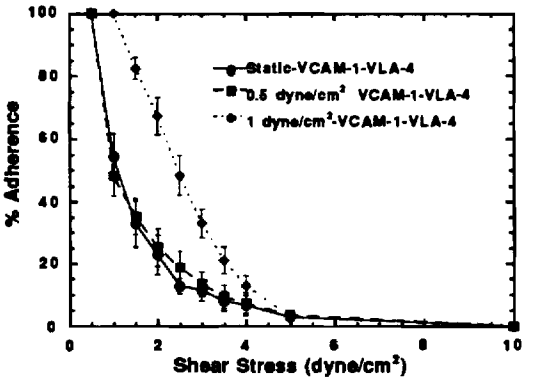

Figure 5. Effect of attachment shear stress on the percent of sickle cells remaining adherent to endothelium via $\alpha_4\beta_1$/VCAM-1 as detachment shear stress is increased.

3. Significance

The Role of Viral Infection in Sickle Cell Adherence

The data of Figure 1 provides clear evidence that double stranded RNA or double stranded RNA viruses can induce endothelial cell VCAM-1 expression and sickle cell adherence via $\alpha_4\beta_1$/VCAM-1. The clinical association of febrile events (15) with acute pain episodes in sickle cell patients suggests a role for infection in the

development of vaso-occlusive pain episodes. Although research has focused on bacterial infection in sickle cell anemia (16,17), viral infection also appears to play a role in causing complications in sickle cell disease. During confirmed cases of infection by the RNA virus Influenza B, acute pain episode have been reported (18), and viral infections are thought to precipitate such complications as acute chest syndrome (19). Infection is known to activate certain leukocytes causing release of cytokines such as type I interferons and TNF-α, and leading to the development of an antiviral environment in the attacked host (20,21). Many sickle cell patients have been clinically observed to have consistently elevated cytokine levels (22). The release of these agents within the vascular system, specifically TNF-α, can lead to increased endothelial surface expression of cellular adhesion molecules, which are then thought to play a role in sickle erythrocyte-endothelial adhesion (6). Viral infection may therefore directly increase adherence through the effects of double stranded RNA on vascular endothelial cells and indirectly by leukocyte activation. Both of these pathways, induced by viral infection, may lead to an activated vascular state that favors high levels of sickle erythrocyte-endothelial adherence and promotes vaso-occlusive complications.

Biophysical Characterization of Sickle Adherence Pathways

Adherence of sickle erythrocytes is complicated and modulated by receptors on sickle erythrocytes and endothelial cells; plasma proteins and blood-borne agonists; and local hemodynamic forces. At least four different adherence pathways have been identified (2,3,5,6,8,14) an adherence is different to large vessel and microvascular endothelium (4).

It is possible that inhibition or reversal of sickle cell adherence to the endothelium could attenuate the ischemic tissue and organ damage associated with sickle cell pain episodes. However, the multiple and different adherence pathways of sickle cell adherence make it difficult to predict which pathway, if any, predominates in the vascular milieu.

The parallel-plate flow chamber adherence assays we have utilized are uniquely suited to studies designed to compare the relative tenacity of competing adherence pathways. As shown in Figures 2-5, adherence between the two pathways studied to date (thrombospondin and $\alpha_4\beta$1/VCAM-1) demonstrate clear differences in the level of sickle cell adherence at sequentially increasing shear stress. Most notably, even though the initial attachment of sickle cells via thrombospondin or $\alpha_4\beta$1/VCAM-1 is essentially equivalent, sickle cells attached to the endothelium via thrombospondin are more readily detached from the endothelium. In fact, at 4 dynes/cm^2 shear stress, essentially all of the sickle cells attached via thrombospondin are removed. In contrast, more than 15% of the cells attached via $\alpha_4\beta$1/VCAM-1 remain adherent at 4 dynes/cm^2. Thus, not only do more sickle cells remain adherent at each shear stress via $\alpha_4\beta$1/VCAM-1, a significant fraction of these cells remain tenaciously adherent to the endothelium at a shear stress level that would be observed in the post-capillary venules.

Biophysical characterizations of sickle cell adherence, such as those presented here, provide insights into the conditions under which sickle cell adherence via specific mechanisms occurs. These data, generated using human vascular endothelial cells under hemodynamic conditions which mimic those *in vivo*, will be useful in identifying the ability of specific pathways to promote adherence under a variety of vascular conditions that might arise in response to infection, thrombosis or other hematopoietic stresses which may increase the level or tenacity of sickle cell adherence. Then, with this more complete understanding of sickle cell adherence under a wide variety of conditions, effective anti-adhesion therapeutics can be more efficiently developed to inhibit or reverse sickle cell adherence and the attendant vascular complications.

4. Plans

Biophysical Characterization of Adherence Pathways - Strength of Adherence

The data of Figures 2-5 suggest that differences in the tenacity of sickle cell adherence observed under shear conditions is mediated by specific receptors and ligands which are sensitive to kinetics (contact time), hemodynamic forces (shear stress), and available contact area (steric considerations). Our immediate plans are to characterize the tenacity of sickle cell adherence to endothelium via the other two adherence pathways (mediated by high molecular weight von Willebrand factor multimers bridging receptors on sickle erythrocytes and endothelial cells and sickle cell phorbol ester activated $\alpha_4\beta_1$, binding to fibronectin on endothelial cells). Shear stress wash out curves will be generated as for the other two pathways (Fig 2,3) and the effect of attachment conditions on tenacity will be qualified (Fig 4,5).

Note that the above experiments involve studying individual adherence pathways under isolated conditions. Thus, this initial series of experiments will indicate two things. First, by quantifying the number density of adherent red cells under otherwise identical conditions, the relative level of adherence via each pathway can be obtained. Secondly, the wash out curves indicate the pathway(s) which provide for the strongest sickle cell adherence. It is likely that both the absolute number of adherent cells and the tenacity of the adherence are important for clinically relevant adherence in vivo.

Biophysical Characterization of Adherence Pathways - Kinetics of Adherence

In conjunction with the detachment assays, which primarily provide data on the strength of sickle cell adherence, we plan to initiate studies to measure the rate (kinetics) of sickle cell adherence to endothelium via the various pathways. For these studies we have designed and constructed a cone-and-plate viscometer to be used to observe the kinetics of sickle cell attachment under varying conditions of activation. Figure 6 is a schematic diagram of our dynamic assay to measure red cell attachment kinetics. This system has been recently tested and can provide a constant and continuous shear stress for up to 48 hours without endothelial cell loss or contamination. Our experimental design will be to subject a suspension of sickle or normal erythrocytes to a shear stress in the range of 0.1 - 10 dynes/cm^2 and visually monitor and record the level of adherence to endothelium in response to manipulations of the biochemical or biophysical environment. For example, the injection ports allow for introduction of agonists (e.g. TNF-α, Poly(I:C), phorbol ester, etc.) and/or adhesive proteins (thrombospondin, von Willebrand factor). Then, the level of adherence in response to these manipulations will provide data on the kinetics via a specific pathway. These data will indicate the rate of sickle adherence induced by a specific alteration in the vascular milieu such as double stranded RNA virus infection, cytokine release, or high molecular weight von Willebrand factor release. More importantly, this system will be useful to test anti-adherence compounds (such as RGD peptides) for their ability to dislodge adherent cells in a flow environment. Thus, the attachment kinetics data will complement the strength of adherence data and provide a more complete picture of the sickle cell adherence pathways involved *in vivo* under a variety of normal and pathological conditions.

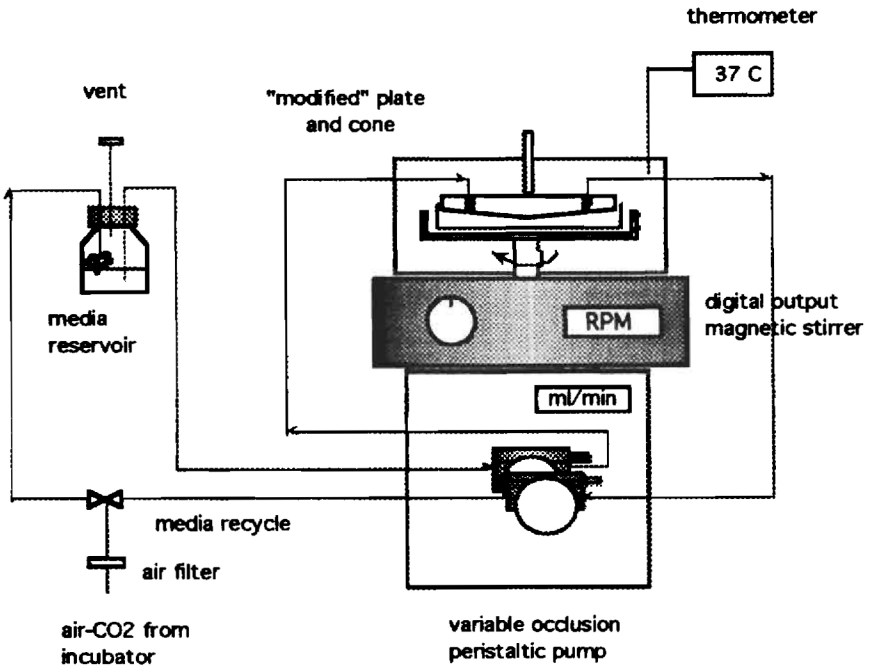

Figure 6. Cone-and-plate apparatus to measure the kinetics of sickle cell adherence. Note that the injection ports allow for the circulation of agonists, adhesive proteins, or other effectors of cell adherence or disadherence.

Literature Cited

1. Kaul DK, ME Fabry, RL Nagel. Microvascular sites and characteristics of sickle cell adhesion to vascular endothelium in shear flow conditions: Pathophysiological implications. Proc Natl Acad Sci, USA 86:3356; 1989.
2. Wick TM, JL Moake, MM Udden, SG Eskin, DA Sears and LV McIntire. Unusually large von willebrand factor multimers increase adhesion of sickle erythrocytes to endothelial cells under controlled flow. J Clin Invest 80:905; 1987.
3. Wick TM, JL Moake, MM Udden, and LV McIntire. Unusually large von willebrand factor multimers preferentially promote young sickle and non-sickle erythrocyte adhesion to endothelial cells, Am J Hematology, 42:284; 1993.
4. Brittain HA, JR Eckman, and TM Wick. Sickle erythrocyte adherence to large vessel and microvascular endothelium under physiologic flow is qualitatively different. J Lab Clin Med 19:538; 1992.
5. Brittain HA, JR Eckman, RJ Howard TM Wick: Thrombospondin from activated platelets promotes sickle erythrocyte adherence to human microvascular endothelium under physiologic flow: A potential role for platelet activation in sickle cell vaso-occlusion. Blood, 81:2137; 1993.
6. Swerlick RA, JR Eckman, A Kumar, M Jeitler, and TM Wick. $\alpha_4/ß_1$-integrin expression on sickle reticulocytes: vascular cell adhesion molecule-1-dependent binding to endothelium, Blood, 82:1891; 1993.
7. Wick TM, PA Smolinski, MK Offermann, and JR Eckman. Synthetic double-stranded RNA increases adherence of sickle red blood cells to human umbilical vein endothelial cells via $\alpha_4/ß_1$-vascular cell adhesion molecule-1 pathway, Blood, 82:352a; 1993.
8. Kumar A, JR Eckman, RS Swerlick, and TM Wick. Sickle erythrocytes stimulated with phorbol ester adhere to endothelium via activated $\alpha_4\beta_1$ integrin on sickle reticulocytes and fibronectin on endothelium. Blood 84:408a; 1994.
9. Diggs LW. Sickle cell crisis. Amer J Clin Pathol 44:1; 1965.
10. Yang J, Hagan MK, Offermann MK: Induction of IL-6 gene expression in Kaposi's sarcoma cells. J Immunol 152:943; 1994.
11. Yang J, Xu Y, Zhu C, Hagan MK, Lawley T, Offermann MK. Regulation of adhesion molecule expression in Kaposi's sarcoma cells. J Immunol 152:361, 1994.
12. Einhorn S, Eldor A, Vlodavsky I, Fuks Z, Panet A. Production and characterization of interferon from endothelial cells. J Cell Physiol 122:200; 1985.
13. Offermann MK, Medford R. Induction of VCAM-1 gene expression by double stranded RNA occurs by a p68 kinase-dependent pathway in endothelial cells. Clin Res 412:262A; 1993.
14. Sugihara K, T Sugihara, N Mohandas, RP Hebbel, Thrombospondin mediates adherence of $CD36^+$ sickle reticulocytes to endothelial cells. Blood, 80:2634-2642;1993.
15. Baum KE, Dunn DT, Maude GH, Serjeant GR: The painful crisis of homozygous sickle cell disease. Arch Intern Med 147:1231; 1987.
16. Barrett-Connor E: Bacterial infection and sickle cell anemia. Medicine 50:97, 1971.
17. Winkelstein JA: Pneumococcal infections in sickle cell disease. J Pediat 91:521, 1977.
18. Turner EA, Thompson HD, Reddy CM, South MA, Garrett-Ellis BR, Mirkovic RR: Sickle cell disease with complicated influenza B virus infection. J Natl Med Assoc 84:524, 1992.
19. Poncz M, Kane E, Gill FM: Acute chest syndrome in sickle cell disease: Etiology and clinical correlates. J Pediatr 107:861, 1985.
20. Goldfeld AE, Maniatis T: Coordinate viral induction of tumor necrosis factor α and interferon β in human B cells and monocytes. Proc Natl Acad Sci USA 86:1490, 1989.
21. Wathelet MG, Berr PM, Huez GA: Regulation of gene expression by cytokines and virus in human cells lacking the type-1 interferon locus. Eur J Biochem 206:901, 1992.

22. Francis RB, Haywood LJ: Elevated immunoreactive tumor necrosis factor and interleukin-1 in sickle cell disease. J Natl Med Assoc 84:611, 1992.

5. Human Subjects

a. General Guidelines

i. Proposed Use

Patients with sickle-cell syndromes (HbSS, HbSC, HbS β-thalassemia) not receiving anticoagulant therapy and without evidence of pregnancy, obvious infection, thromboembolic disease or liver disease will be eligible for this study. Patients will be studied once in pain crisis and twice during asymptomatic periods. An age and sex matched population of normal black individuals will serve as a control population. Approximately twenty patients and twenty control subjects, aged eighteen or older, will be studied annually. Ten milliliters of blood will be drawn by venipuncture for each experiment.

ii. Specimen Usage

None of the data from the experiments will be used for diagnosis or treatment of specific individuals.

iii. Patient Recruitment

Patients from the Georgia Comprehensive Sickle Cell Center or the in-patient service at Grady Memorial Hospital, Atlanta, GA and hospital staff will be recruited by Dr. James R. Eckman, M.D. Subjects will agree to participate in this study by signing a consent form approved by Georgia Tech and Emory University School of Medicine IRBs. The consent form explains the nature of the study, the details of blood collection, risks associated with drawing blood, the availability of personnel to discuss the results of the study, the assurance of anonymity, and the ability to withdraw from the study at any time without penalty or loss of benefits.

iv. Potential Risks

The risks of drawing blood are minimal and include slight pain, bruising, and infection at the site of puncture. No viable alternative for drawing human blood exists.

v. Procedures to Minimize Risk

Patient confidentiality is be ensured by assigning an alphanumeric code to each patient studied to be used when all data is reported. Blood will be drawn at Grady Hospital under the supervision of Dr. James R. Eckman, director of the Georgia Comprehensive Sickle Cell Center. Dr. Eckman will be available to answer questions and to arrange for emergency medical care if a medical problem develops during the course of this study.

vi. Justification

The risk of blood drawing is minimal compared to potential benefits of a better understanding of clotting abnormalities in sickle cell syndromes and their relationship to pain crisis.

b. Gender and Minority Inclusions

Study subjects will be patients diagnosed with sickle cell syndromes as defined above. These patients will primarily be of African descent, however no patients will be included or excluded on the basis of race. The study population will consist of approximately equal numbers of men and women. Exclusion criteria will be solely based on medical criteria as described above. Control subjects (volunteers without hemoglobinopathies) will be age, sex, and race-matched. These volunteers are recruited from the hospital staff at Grady Memorial Hospital in Atlanta.

6. Vertebrate Animals

None.

7. Publications (from this project)

a. Journal Articles

1. Smolinski, P.A., M.K. Offermann, J.R. Eckman, and T.M. Wick. "Synthetic Double Stranded RNA Increases Sickle Red Blood Cell Adherence to Endothelium via a VCAM-1/VLA-4 Pathway," Blood (In review).

b. Abstracts and Meeting Presentations

1. Brown, M., J.R. Eckman and T.M. Wick. "Sickle Erythrocytes Promote Increased Expression of Cell Adhesion Molecules on Human Umbilical Vein Endothelial Cells," 1993 Annual Meeting of the American Institute of Chemical Engineers, St. Louis, Missouri (November 1993).
2. Wick, T.M., P.A. Smolinski, M.K. Offermann, and J. R. Eckman. "Synthetic Double-Stranded RNA Increases Adherence of Sickle Red Blood Cells to Human Umbilical Vein Endothelial Cells via a_4b_1 - Vascular Cell Adhesion Molecule-1 Pathway," Blood, **82**:352a (1993). Annual Meeting of the American Society of Hematology, St. Louis, MO (December 1993).
3. Wick, T.M,. M.D. Brown, and J.R. Eckman. "Sickle Red Blood Cells Induce Expression of Cell Adhesion Molecules on Human Umbilical Vein Endothelial Cells," Blood, **82**:352a (1993). Annual Meeting of the American Society of Hematology, St. Louis, MO (December 1993).
4. Brown, M.D., J.R. Eckman, and T.M. Wick. "Increased Expression of Human Umbilical Vein Endothelial Cells Adhesion Molecules in Expressed by Sickle Cells," 19th Annual Meeting of the National Sickle Cell Program, New York, NY (March 1994).
5. Smolinski, P.A., M.K. Offermann, J.R. Eckman, and T.M. Wick. "Increased Adherence of Sickle Erythrocytes to Human Endothelial Cells Incubated with Double-Stranded RNA Occurs via an a_4b_1-Vascular Cell Adhesion Molecule-1 (VCAM-1) Pathway," 19th Annual Meeting of the National Sickle Cell Program, New York, NY (March 1994).
6. Smolinski, P.A., M.K. Offermann, J.R. Eckman, and T.M. Wick. "Double Stranded RNA Mediates Sickle Erythrocyte a_4b_1-Endothelial VCAM-1 Adhesion: A Potential Role for Viral Infection in Sickle Cell Vaso-Occlusion," Annals of Biomedical Engineering, **22**:29;1994 (suppl 1). 1994 Annual Fall Meeting of the Biomedical Engineering Society, Tempe, AZ (October 1994).
7. Brown, M.D., J.R. Eckman, and T.M. Wick, "Endothelial Activation upon Incubation with Sickle Cells," Annals of Biomedical Engineering, **22**:33;1994 (suppl 1). 1994 Annual Fall Meeting of the Biomedical Engineering Society, Tempe, AZ (October 1994).
8. Smolinski, P.A., J.R. Eckman, and T.M. Wick. "*In Vitro* Comparison of Known Mechanisms of Sickle Erythrocyte Adherence to Endothelium: Biophysical Studies to Predict the Relative Importance of Competing Adherence Pathways *In Vivo*," Blood, **84**:407a (1994). 1994 Annual Meeting of the American Society of Hematology, Nashville, TN (December 1994).
9. Smolinski, P.A., J.R. Eckman, and T.M. Wick. "Biophysical Studies to Predict the Relative Tenacity of Receptor Medicated Sickle Erythrocyte Adherence Pathways *In Vitro:* Implications for Physiological Significance in the onset of Vascular Occlusion in Sickle Cell Anemia," 20th Annual Meeting of the National Sickle Cell Program, Boston, MA (March 1995).
10. Brown, M.D., J.R. Eckman, T.M. Wick, "Modulation of Endothelial Cell Adhesion Molecule Expression by Sickle Cells is Mediated Through the Production of Soluble Factor," 20th Annual Meeting of the National Sickle Cell Program, Boston, MA (March 1995).

11. Brown, M.D., J.R. Eckman, and T.M. Wick. "Induction of Endothelial Adhesion Molecule Expression by Sickle Cells: A Possible Mechanism for Increased Vascular Disease," Experimental Biology '95, Atlanta, GA (April 1995).
12. Smolinski, P.A., J.R. Eckman, and T.M. Wick, "Biophysics of Sickle Cell Adherence to Endothelium," ," Experimental Biology '95, Atlanta, GA (April 1995).
13. Smolinski, P.A., J.R. Eckman, and T.M. Wick. "Biophysical Studies to Predict the Relative Tenacity of Receptor Medicated Sickle Erythrocyte Adherence Pathways *In Vitro:* Implications for Physiological Significance in the Onset of Vascular Occlusion in Sickle Cell Anemia," 1995 ASME/AIChE/ASCE/BMES Summer Bioengineering Conference, Beaver Creek, CO (June 1995).

7. Inventions and Patents

None.

PROGRESS REPORT *(Personnel and Study Subjects)*

GRANT NUMBER
1 P60 HL48482-03

All Personnel for the Current Budget Period
and Any Planned Changes in Personnel for the Next Budget Period
Use two sections. In the first section list *All Current Personnel*. In the second section list *Planned Personnel Changes*.

Name	Degree(s)	SSN	Role on Project *(e.g., PI, Res. Assoc.)*	Date of Birth *(MM/DD/YY)*	Annual % Effort
Current Personnel					
Timothy M. Wick	Ph.D.	505-94-2891	PI	07/09/61	20%
James R. Eckman	M.D.	471-48-8946	Co-Investigator	08/25/43	5%
Paula A. Smolinski	B.S.	274-66-1040	Graduate Student	12/02/69	100%
Richard Montez	M.S.	639-24-5702	Graduate Student	10/24/67	100%
Marena Brown	M.S.	498-80-4556	Graduate Student	05/27/67	100%
Planned Changes					
Additions					
Research Associate	B.S.				20%

Provide the number of subjects enrolled in the study *to date* according to the following categories. (See Page 8 for definitions.)

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown	TOTAL
Female			38		3	1	42
Male			23		2	1	26
Unknown							
TOTAL			61		5	2	68

CHECKLIST

GRANT NUMBER

1PO HL48482-03

Check the appropriate boxes and provide the information requested. Make this page the last page of the signed original of the applicatio

1. ASSURANCES/CERTIFICATIONS

The following assurances/certifications are made by checking the appropriate boxes and verified by the signature of the OFFICIAL SIGNING FOR APPLICANT ORGANIZATION on the FACE PAGE of the application. Descriptions of individual assurances/certifications begin o page 9 of Specific Instructions.

a. Human Subjects *(Complete Item 7 on the Face Page)*

[X] Full IRB Review [ ] Expedited Review

Use of Human Subjects [ ] Change [X] No Change Since Previous Submission

b. Vertebrate Animals *(Complete Item 8 on the Face Page)*

Use of Vertebrate Animals [ ] Change [ ] No Change Since Previous Submission

c. Inventions and Patents *(Complete Item 11 on the Face Page)*

d. Debarment and Suspension [X] No [ ] Yes *(Attach explanation)*

e. Lobbying

With Federal appropriated funds [ ] No

With other than Federal appropriated funds [ ] No [ ] Yes

(If "yes", see page 13, and attach Standard Form LLL, "Disclosure of Lobbying Activities," to the application behind the Checklist.)

f. Delinquent Federal Debt [X] No [ ] Yes *(Attach explanation)*

g. Misconduct in Science (Form PHS 6315) [X] Filed [ ] Not Filed

If filed, date of Initial Assurance or latest Annual Report 2125193

h. Civil Rights	i. Handicapped Individuals	j. Sex Discrimination	k. Age Discrimination
Form HHS 441	Form HHS 641	Form HHS 639-A	Form HHS 680
[X] Filed	[X] Filed	[X] Filed	[X] Filed
[ ] Not Filed	[ ] Not Filed	[ ] Not Filed	[ ] Not Filed

2. PROGRAM INCOME *(See Instructions, Page 16)*

All applications must indicate (Yes or No) whether program income is anticipated during the period(s) for which grant support is requested. [X] No [ ] Yes. If "Yes" use the format below to reflect the amount and source(s) of anticipated program income.

Budget Period	Anticipated Amount	Source(s)

3. INDIRECT COST

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office, or, in the case of forprofit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office. Indirect costs will not be paid on foreign grants, construction grants, grants to Federal organizations and grants to individuals, and usually not on conference grants. Follow any additional instructions provided for Research Career Development Awards, Institutional National Research Service Awards, and specialized grant applications.

[ ] DHHS Agreement Dated: _____ [ ] No Indirect Costs Requested

[X] No DHHS Agreement, but rates established with Office of Naval Research Date _____

CALCULATION*

Enter proposed budget period:

Amount of base $ 86,439 × Rate applied 0.40 % = Indirect costs $ 34,576

Add to total direct costs from form page 2 and enter new total on Face Page, item 10b

*Check appropriate box(es):

[ ] Salary and wage base [X] Modified total direct costs base [ ] Other base (Attach explanation)

[ ] Off-site, other special rate, or more than one rate involved *(Attach explanation)*